

IMMUNOLOGICAL ASPECTS OF SEVERE NEONATAL INFECTION WITH GROUP B STREPTOCOCCI

With special reference to maternal serum
antibodies and possible measures for the
prevention of neonatal infection

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The thesis is based on the following publications which will be referred to by their Roman numerals:

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- III Grubb R, Christensen KK, Christensen P, Lindén V: Association between maternal Gm allotype and neonatal septicaemia with group B streptococci. J Immunogenetics 9:143-147, 1982.
- IV Lindén, V: Mouse-protective effect of rabbit anti R-protein antibodies against group B streptococci type II carrying R-protein. Lack of effect on type III carrying R-protein. Acta path. microbiol. immunol. scand. Sect. B. In press.
- V Lindén V, Christensen KK, Christensen P: The occurrence of R-protein among isolates of group B streptococci from human sources. Acta path. microbiol. immunol. scand. Sect. B. In press.
- VI Lindén V, Christensen KK, Christensen P: Correlation between low levels of maternal IgG antibodies to R-protein and neonatal septicemia with group B streptococci carrying R-protein. Int. Arch Allergy appl. Immun. In press.
- VII Lindén V, Christensen KK, Christensen P: Low levels of antibodies to surface antigens of group B streptococci in commercial IgG preparations. Int. Archs Allergy appl. Immun. 68:193-195, 1982.
- VIII Christensen KK, Dahlander K, Lindén V, Svenningsen N, Christensen P: Obstetrical care in future pregnancies after fetal loss in group B streptococcal septicemia. A prevention program based on bacteriological and immunological follow up. Europ. J. Obstet. Gynec. reprod. Biol. 12:143-150, 1981.



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Abbreviations

cpm = counts per minute

CSF= cerebrospinal fluid

ELISA = enzyme linked immunosorbent assay

FHR = fetal heart rate

GBS= group B streptococci

Gm= genetic marker (on the IgG molecule)

PMN= polymorphonuclear leukocytes

TCA= trichloroacetic acid

Review of Literature

Retrospect

Infections with streptococci in man and animals have been recognized for a century. The name Streptococcus was first used by Rosenbach in 1884, describing a chain-forming coccus, which had been isolated from suppurative lesions in man. Nocard and Mollereau (1887) described the isolation of a streptococcal strain from the milk of a cow with mastitis and - to complete the Koch-Henles postulates - the experimental production of bovine mastitis with this strain. They named the bacteria Streptococcus mastitidis contagiosae. The study was followed by many reports concerning the isolation of chain-forming streptococci from a variety of healthy human beings and animals, from milk and various milk products and from other sources. These strains, now recognized as group B streptococci (GBS), were shown to cause epidemic outbreaks of mastitis in cattle. Infections with this bacterium is still a serious economic problem in dairy farming.

In 1903, Schottmüller described seven cases of human infections in which pathogenic streptococci were isolated from various locations. He noticed differences in colony form, colour and hemolysis among streptococci cultured on blood-agar. Hence, Schottmüller was the first to describe hemolysis of red blood cells by streptococci. Brown suggested, in 1919, the terms α , β and γ streptococci and found streptococci of both human and bovine origin belonging to the β -hemolytic form.

In the 1920s, one of the most important problems to be tackled in combating β -hemolytic streptococci was to differentiate between seemingly apathogenic bovine β -streptococci occurring in cow milk and hemolytic streptococci of human origin causing epidemic out-breaks of scarlatina when spread by milk. The close similarity between β -hemolytic streptococci of human and bovine origin led to extensive studies of the organisms.

In 1922 Ayers and Rupp found that sodium hippurate was hydrolyzed by hemolytic streptococci of bovine but not of human origin. Possibly a similar reaction was described already in 1895 by Crisafulli for Streptococcus erysipelatis (Ayers and Rupp, 1922). A method for serological classification by precipitin reaction was described by Hitchcock in 1924. He found the precipitating streptococcal substance to be practically free from protein. This carbohydrate substance was later studied and named by Lancefield (1928) as C-substance. It formed the basis

for a serological grouping system by which the majority of the β -hemolytic streptococci pathogenic to man was found to belong to group A and most of the bovine strains to group B (1933).

Stableforth (1932, 1937) differentiated GBS into three serological "types" and a number of subtypes. He used the precipitin and agglutination techniques of Griffith (1926) and presumed that the "type-specific" antigens were proteins. However, in 1934 Lancefield (1934a) reported on three type-specific polysaccharides in GBS, subdividing GBS into types I, II and III. This scheme was subsequently enlarged to four types: Ia, Ib, II and III (1938) and the type-specificity of the capsular polysaccharides was to some extent confirmed by "protective antibody studies" (1941). In 1971, Wilkinson and Eagon added a new serotype called Ic, which is now officially recognized.

In 1934, Hare reported that streptococcal strains isolated from vagina of women with minor post partum infections did not grow in human blood while strains isolated from women with septicemia did. Furthermore, the former strains were less virulent in mice. Subsequently, Hare and Colebrook (1934) showed that β -hemolytic streptococcal strains from mild puerperal infections and the majority of "saprophytic" strains occurring in vaginal specimens were similar to bovine strains. Finally, in 1935 Lancefield and Hare reported that the majority of β -hemolytic streptococci isolated from the birth canal, and which did not bring about active infections, belonged to group B resembling the strains causing bovine mastitis, whereas streptococci of group A caused the severe puerperal infections. Already 100 years earlier, in 1839, these puerperal infections were described by a Swedish obstetrician, Per Gustaf Cederschjöld, who also described the use of chlorine water to prevent the spreading of puerperal fever among women in delivery wards (1839). This was ~~eight~~ years before the reports by Semmelweis.

In the 1920s and 30s GBS were considered harmless to man. The first isolation of GBS strains from septicemia following childbirth was described by Hare in 1935. Fry (1938) reported three fatal cases of GBS infection in the puerperium. The first report on septicemia and meningitis in newborns was published by Plummer (1941). During the following decades a large number of different clinical conditions were found to be associated with GBS, although the bacterium was generally considered harmless in man. The case reports concerned meningitis, bacteriaemia, endocarditis, bronchopneumonia, arthritis, peritonitis, wound infections, abscesses, urinary tract infections, lymphadenitis, laryngitis and pharyngitis. GBS infections have also been described in compromised hosts, e.g. in patients suffering from diabetes or neoplastic diseases (reviewed by Jelinkova, 1977; Patterson and Hafeez, 1976; Wilkinson, 1978c).

The association between GBS and serious problems of the neonatal period and the importance of GBS as human pathogens was not recognized until the beginning of the 60s. Hood and coworkers (1961) described the association between GBS and spontaneous abortions, perinatal death, prematurity and severe neonatal illness. Subsequently, Eickhoff and coworkers (1964) reported GBS to be the most frequent single cause of neonatal infections accounting for 25 %. These two studies were followed by a large number of reports on perinatal GBS infections. However, it was not until the 1970s that GBS was firmly established as a major problem in perinatal infections.

The most important source of GBS infection is vaginal colonization in pregnant women. The majority of colonized newborns do not develop an infection. Both colonized infants without infection and those who acquire early onset septicemia and/or meningitis, harbour the GBS serotype of their mothers. The recognition of this fact indicated that the infants were infected either in utero or during passage through the birth canal. Until 1976, it was not clear why only a few per cent of the GBS-colonized children contracted septicemia and/or meningitis. Baker and Kasper (1976a) found that absence of maternal antibodies to type III was associated with GBS type III infection in newborns and suggested that transplacental transfer of maternal antibody protects infants from GBS infection. However, the immunological defense mechanisms involved in the protection against neonatal GBS infections are still not clearly understood. They will be further discussed elsewhere in the thesis.

Bacteriology of group B streptococci (GBS)

According to Bergey's manual (1974), the group B Streptococcus is a member of the genus Streptococcus, which represents the family Streptococcaceae of gram-positive cocci. Synonyms of the species are S. nocardii, S. mastitis contagiosae, S. mastitidis, S. mastitis sporadicae, S. agalactiae contagiosae, S. opportunistus and S. agalactiae (Jelinkova, 1977; Patterson and Hafeez, 1976). However, today S. agalactiae is the most widely accepted nomenclature. All strains of the S. agalactiae are classifiable as belonging to Lancefield's serological group B on the basis of the group specific polysaccharide (C-) substance (Lancefield, 1933). The cocci are 0.6 - 1.2 μm in diameter and occur in chains which are frequently very long and seldomly consist of less than four cells. GBS are easily cultured on horse blood agar at +37°C, aerobically, as well as anaerobically. The colony morphology is characteristic, with a narrow zone of hemolysis (Brown 1920, 1937). Less than 2% of the strains are non-hemolytic but still virulent in man (Anthony and Concepcion, 1975). Colonies on sheep blood agar are larger in diameter, mucoid and exhibit a more narrow zone of hemolysis than do group A streptococci (Baker, 1980). Some GBS strains produce an orange pigment

(Lancefield, 1934b). The distinction between GBS and group D streptococci on the basis of differences in colony morphology is nigh on impossible (Baker, 1980). While other β -hemolytic streptococci do occur in the urogenital tract, the differential distinction between GBS and group D streptococci is the most important. The following laboratory tests have been suggested for preliminary identification of GBS:

	group A streptococci	GBS	group D streptococci	
inhibition by bacitracin	+	-	-	(Facklam et al, 1974)
hydrolysis of sodium hippu- rate broth	-	+	-	(Facklam et al, 1974)
CAMP-testing	-	+	-	(Christie et al, 1944; Munch- -Petersen, 1945)
fermentation of aesculin	-	-	+	(Skadhauge, 1950)
growth on tellu- rite medium	-	grey colonies	black colonies	(Skadhauge, 1950).

Non-hemolytic strains also possess these characteristics. The basic fermentation properties of the species were described by Brown in 1920. Fermentation of lactose has been used to distinguish between bovine and human GBS, the latter thought to be incapable of fermenting lactose (Butter and Moor, 1967).

A higher isolation frequency of GBS from the urogenital tract can be attained by using a selective medium composed of Todd-Hewitt broth containing gentamicin and nalidixic acid (Baker et al, 1975), which prevents overgrowth of other microorganisms in clinical specimens.

The final GBS classification requires serological identification of the polysaccharide. Several methods are now available, i.e. capillary precipitation (Lancefield, 1934a), double diffusion-in-gel (Lancefield and Freimer, 1966), staphylococcal coagglutination (Christensen et al, 1973), immunofluorescence (Romero and Wilkinson, 1974), counterimmunoelectrophoresis (Hill et al, 1975), radiolabelled protein A (Christensen and Christensen, 1978), latex agglutination (Webb and Baker, 1980) and enzyme-linked inhibition assay with monoclonal antibodies (Polin and Kennett, 1980).

GBS are susceptible to many clinically used antimicrobial agents, for example, benzylpenicillin, ampicillin, cefuroxime and other new cephalosporines, clindamycin and erythromycin (Baker CN et al, 1981; Bayer et al, 1982). The minimum inhibitory concentrations (MIC) reported for penicillin are low (Baker CN et al, 1981; Eickhoff et al, 1964) but considerable higher than those registered for group A streptococci. GBS are resistant to nalidixic acid (Baker CN et al, 1981). Tetracycline resistance appears to be frequent among clinical isolates, almost 85 % (Anthony and Concepcion, 1975; Baker et al, 1976b). Resistance to the aminoglycosides is uniform but the combination of penicillin or ampicillin and gentamicin has synergistic activity and accelerates the killing of GBS in vitro (Baker CN et al, 1981). Four % of clinical GBS isolates have been reported penicillin-tolerant (Kim and Anthony, 1981). Penicillin and gentamicin have no synergistic effect on the penicillin-tolerant variants. However, the clinical importance of penicillin-tolerance in GBS is not quite clear although it has been associated with recurring infections (Broughton et al, 1976; Siegel et al, 1981).

Antigenic structure

The group-specific polysaccharide is serologically identical in all GBS strains and part of the cell-wall (Curtis and Krause, 1964). As mentioned above Lancefield (1938) described four serological types of GBS; Ia, Ib, II and III, and characterized the four types as having different polysaccharide antigens. An additional serotype, type Ic, was defined by Wilkinson and Eagon (1971). Type Ic strains contain a polysaccharide immunochemically identical to that found in type Ia strains (Wilkinson, 1975) but carry an additional protein antigen, designated the Ibc protein. The Ibc protein is shared with type Ib strains and infrequently with strains of types II and III. The protein consists of two protein chains, the α - and β -antigens (Bevanger and Iversen, 1981). Strains of types Ia, Ib and Ic share a cross-reactive antigen determinant, designated the Iabc minor polysaccharide (Lancefield et al, 1975). The type-specific antigens are located in the capsule as shown with electron microscopy (Jelinkova and Rýc, 1979; Kasper and Baker, 1979). Serological studies also suggest that the type-related polysaccharide antigens are peripheral to the group B polysaccharide antigen, stereochemically hindering antibodies from binding to the latter (Wilkinson, 1978c). For these reasons the type-specific polysaccharides are sometimes called capsular antigens and presumably impede phagocytosis of the organisms. A new type-specific antigen, probably another polysaccharide antigen, has been described by Perch and coworkers (1979). They found that 20 % of their annual 10 % non-typable strains reacted with antisera raised against a nontypable GBS strain. This indicates that a new type, type IV, might have to be added.

The classical method for extraction of polysaccharide antigens from GBS for serogrouping and serotyping includes treatment of whole organisms with hot HCl (Lancefield, 1933). Additional purification is necessary to separate group- and type-specific antigens. Thus, the type I polysaccharide (Wilkinson, 1975), the type II (Lancefield and Freimer, 1966) and the type III polysaccharide (Russel and Norcross, 1972) have been purified by using alcohol fractioning or ion exchange chromatography. The HCl extracted type-specific antigens are incomplete; they are low molecular weight substances, containing various quantities of three monosaccharides - galactose, glucose and glucosamine - in repeating units of a "core" structure (Baker et al, 1976a; Kasper et al, 1978; Lancefield and Freimer, 1966; Russel and Norcross, 1972; Wilkinson, 1975). The group-specific polysaccharide is composed of rhamnose, N-acetylglucosamine and galactose (Curtis and Krause, 1964). L-rhamnose is the major component of the antigenic determinant. The isolation of more "complete" typespecific antigens can be accomplished by gentler methods such as extraction with cold trichloroacetic acid (TCA) or neutral buffer treatment of whole cells (Baker et al, 1976a; Freimer, 1967; Kane and Karakawa, 1977; Kasper et al, 1978; Lancefield 1933, 1972; Lancefield and Freimer, 1966; Tai et al, 1979; Wilkinson, 1975). These preparations (TCA and "native" antigens) of all serotypes of GBS contain acidlabile sialic acid, which occupies terminal positions on the side chains of the core structure (Baker and Kasper, 1976b). Acid hydrolysis of the TCA (native) antigen of all serotypes results in degradation to core fragments that are immunologically identical to the HCl polysaccharides and contain no sialic acid (Lancefield, 1972). Sialic acid is also a major constituent of the polysaccharide antigens of *E. coli* carrying K1 antigen (Kasper et al, 1973; Robbins et al, 1974), a wellknown pathogen in neonatal meningitis, and of *N. meningitidis* group B (Kasper et al, 1973). Sialic acid has been suggested as a virulence factor, important for the invasion of the meninges of neonates (Baker and Kasper, 1976b; Baker et al, 1976a). The incomplete type III antigen, which is structurally identical to *S. pneumoniae* type 14-polysaccharide (Jennings et al, 1980), is obtained by cleavage of all the labile sialic acid end groups of the native polysaccharide antigen of GBS type III.

Lancefield's classification (Lancefield et al, 1975) of serological types depends on passive protection of mice with antibodies directed against the specific antigens, and is defined in these terms. Specific antibodies directed either against polysaccharides or protein antigens of a single strain were demonstrated to protect mice against infection with streptococci containing these antigens, i.e. the polysaccharide antigens Ia, Ib and II and the Ibc protein. Antibody to the Iabc minor carbohydrate antigen, which has yet not been chemically defined, was also protective (Lancefield et al, 1975). On the other hand, antibodies against the group antigen of GBS are not

protective (Lancefield et al, 1975). For several years, it was not possible to show that antibodies to the type III antigens were protective in mice since type III GBS was not believed to be pathogenic in mice (Baker, 1977). Recently, however, Baltimore and coworkers (1979) were able to apply the mouse protection model to GBS type III. Two more protein antigens, R and X, have been added to Lancefield's type classification. The X-antigen shows random variation in vitro when it occurs with polysaccharide III antigen and probably also with the other GBS polysaccharide antigens (Jensen, 1980a). Strains carrying the X-antigen seem almost exclusively related to the bovine reservoir and may be of some epidemiological significance among the cattle (Jensen, 1980a; Pattison et al, 1955a, b). In a study by Jensen (1980a) the X-antigen was demonstrated in more than 50 % of the cattle examined and the "pure" X-strains in 14%, whereas the protein antigen R was present in only 2 %. The X-antigen, in contrast to the R-antigen, has so far never been discovered in isolates from the human urogenital tract (Jensen, 1980b). R and X antigens are unstable and generally considered unrelated to virulence (Lancefield, 1972; Patterson and Hafeez, 1976). Both human and bovine strains are found among the five serotypes of GBS.

Humoral and cellular immune response to GBS

The first evidence that protective immunity against GBS in man was related to type-specific antibodies was reported by Baker and Kasper (1976a). They demonstrated that mothers of neonates developing GBS type III septicemia/meningitis had low serum levels of antibody to the native capsular polysaccharide antigen, as compared to women colonized with GBS type III but giving birth to healthy infants. Several studies using different methods for determination of type-specific antibodies have confirmed these findings (Baker et al, 1981; Christensen et al, 1980a; Hemming et al, 1976; Stewardson-Krieger et al, 1977; Vogel et al, 1979).

Baker and Kasper (1976a) described an assay with radioactive labelled antigen using a polysaccharide preparation containing both group B and type III determinants. Hemming and coworkers (1976) showed that maternal sera often contain type-specific opsonins which reside in the IgG fraction and that the antibodies cross the placenta as seen by comparing maternal and infant cord sera (Baker et al 1977, 1981). With the use of chemiluminescence with normal human neutrophils they also demonstrated the presence of type-specific opsonins. Wilkinson developed a radioimmunoassay for measuring antibodies to all five serotypes (Wilkinson and Jones, 1976; Wilkinson, 1978b). She found that polysaccharide antigens extracted by cold TCA detected quantitatively more antibodies than the corresponding antigens extracted with hot HCl. Contrary to Baker and Kasper (1976a) she reported high levels of antibodies in sera from infected infants and

their mothers. These divergent results were explained when Kasper and coworkers (1979) showed that antibodies directed to the native antigen were highly opsonic, whereas the correlation between antibodies to the core antigen and opsonins was poor. They emphasized the necessity for determining antibodies to native antigens, containing sialic acid, rather than antibodies to core antigens lacking these acid labile determinants. Quantitation of type-specific IgG antibodies against the four major serotypes of GBS by indirect immunofluorescence was described by Vogel and coworkers (1979, 1980). They also reported that a low immunofluorescence antibody titer protected chick embryos against lethal infections with homologous GBS serotypes. Rote and coworkers (1980) described ELISA techniques for the quantitation of antibodies to GBS types II and III. They used antigens extracted with TCA as well as intact bacteria and found that most human sera contained antibodies to intact bacteria but not to TCA antigens. Antibody titers against the intact bacteria correlated well with the opsonic activity measured in a chemiluminescence assay. Quantitation of type-specific IgG antibodies to surface antigens of GBS with a radioimmunological technique utilizing radiolabelled protein A was described by Christensen and coworkers (1980a).

Klesius and coworkers (1973) demonstrated that specific opsonins were necessary for phagocytosis of serotype Ia organisms. Specific antibodies, but not complement, was required for opsonization of serotypes Ia, Ib and Ic as measured by the uptake of radiolabelled GBS by macrophages from rabbit alveoles (Anthony, 1976). With an "opsonophagocytic" assay requiring human polymorphonuclear leukocytes (PMN), immune serum and complement for optimal killing of GBS, Baltimore and coworkers (1977) demonstrated that homologous rabbit antiserum prepared to each of the five GBS serotypes was opsonic in high titers. The protective antibodies in the mouse-protection model used by Lancefield's group (1975) appear to be identical to the "opsonophagocytic" antibodies reported on by Baltimore (1977). In accordance with these findings, a good correlation between an in vivo mouseprotection model and an in vitro bactericidal assay has been shown for type Ia (Stewardson-Krieger et al, 1977). The protection could mainly be ascribed to the IgG class of human sera but also to the IgM class. Opsonic activity was partially dependent on heat-labile serum factors, presumably complement. Chemiluminescence for evaluation of GBS type III opsonins in human sera was described by Anderson and coworkers (1980); the total chemiluminescence activity correlated well with antibody concentration, phagocytic and bactericidal indices.

It is interesting that GBS type Ib, pretreated with subinhibitory concentrations of substances interfering with peptidoglycan synthesis (penicillin, other β -lactam antibiotics, vancomycin) are hypersensitive to the bactericidal activity of human PMN (Horne and Tomasz, 1980), whereas antimicrobials interfering

with protein synthesis do not induce such increased sensitivity. The synergistic action of β -lactam antibiotics and antibody-mediated phagocytosis on GBS is presumable of clinical relevance in eliminating the invading bacteria.

Strains of GBS type II and III do not appear to be uniformly susceptible to opsonization by antibody-containing human sera (Shigeoka et al, 1979a). Resistant organisms appear to require antibody to both the typespecific capsular polysaccharide antigen and to an additional antiphagocytic factor, the nature of which is unknown. Since trypsin treatment produced significantly enhanced opsonization of resistant GBS strains, the antiphagocytic factor may be a protein.

Fischer and coworkers (1981) studied human serum opsonins to GBS type III in an "opsonophagocytic" assay and found type III strains to differ in susceptibility to different human sera. After treatment with neuraminidase, the resistant strains were more susceptible to opsonization, suggesting that sialic acid may function as an antiphagocytic factor in GBS. Santos and his group (1982) also found human isolates of serotypes Ia, II and III, with strain-specific differences in opsonic requirements within the same serotype, to exhibit differences in virulence in an animal model. Resistance to opsonization with homologous rabbit antisera was found to correlate closely to increased virulence for four of 21 clinical isolates of type III in a mouse model (Lindén et al, 1982, unpublished observations). Such differences in strain virulence and opsonic requirements must be considered in the development of active or passive immunotherapy for these severe neonatal infections.

Adults exposed to natural bacterial infection with GBS develop significant increases in antibody within 14 to 21 days (Baker et al, 1981). However, there is little known about the antibody response in neonates infected with GBS. None of 86 neonates with early onset type III GBS infection studied (Baker et al, 1981), showed a detectable increase in the antibody level in sera collected during convalescence. In contrast, some of the infants with late onset meningitis showed increases in the type III antibody level during convalescence. However, the levels decreased within 8-14 weeks to levels corresponding to those detected during acute illness. This could indicate that antibodies produced by young infants following type III meningitis are of IgM class. On the other hand, infants with GBS bone or joint infections uniformly exhibited significant rises in serum antibody concentrations after recovery (Baker et al, 1977). In a recent study of neonatal pneumonia, caused by GBS, without concurrent septicemia, two infants showed a definite immune response against the GBS type isolated; one of these may have responded during intrauterine life (Christensen et al, submitted).

It is evident that some neonates do not develop infection despite very low levels of maternally acquired antibodies to GBS and exposure to the bacteria at birth. This finding suggests the importance of differences in virulence among GBS serotype III strains and differences in neonatal host defence (Baker et al, 1981). The PMN of healthy neonates generally appear to have a phagocytic and bactericidal activity similar to those of adults. However, abnormalities in the bactericidal activity of PMN are found in stressed and/or infected infants (Shigeoka et al, 1979b). A tenfold greater bactericidal survival was evident after exposure of GBS to PMN from infected infants compared with adult controls. Becker and coworkers (1981) demonstrated that PMN phagocytes from newborns had a significantly lower mean bactericidal effect on GBS type III than cells from adults. The presence of subtle abnormalities may possibly contribute to the high mortality rate observed with GBS infection in the neonate (Shigeoka et al, 1979a).

Role of complement in opsonization of GBS

The involvement of the complement system in host defense to GBS has been and still is puzzling. In 1973, Klesius and coworkers reported that a thermolabile plasma-factor enhanced the activity of PMN in phagocytic studies with GBS. On the other hand the opsonic activity of immune serum in a system with macrophages was found to be heat stable by Anthony (1976). In 1974, it was reported that binding of complement by GBS type Ia increased the phagocytic activity; the complement was activated via the classical or alternative pathway, but seldom by both simultaneously (Mathews et al, 1974). Significant enhancement of opsonization of types I and II strains with "whole fresh human complement" was said to suggest that the alternative pathway may not play a major role in opsonization of such strains (Hemming et al, 1976). This finding was confirmed with an "opsonophagocytic" assay (Baltimore et al, 1977) and with a chemiluminescence and a bacterial uptake technique (Shigeoka et al, 1978a). Their studies indicated the requirement of antibody in opsonization of GBS and a significant role of the classical complement pathway. In contrast, Andersson and coworkers (1980) reported that type-specific antibody in addition to heat labile serum factors, including those of the alternative pathway, contribute to opsonization of GBS type III. Studies by Edwards and coworkers (1980) indicated that low levels of antibody utilize the classical pathway, but higher levels of specific antibody recruit the alternative pathway. Thus, it was shown that native capsular polysaccharide antigen of type III contains terminal sialic acid residues on each repeating unit that masks all end-group galactospyranose residues and prevents complement activation by the alternative pathway in adult human serum in the absence of type-specific antibody. Sialic acid increases the affinity of $\beta 1H$ relative to B for C3b and this results in blocking formation of the alternative pathway C3 convertase

C3bBb (Fearon, 1978). Hence, sialic acid functions as a non-activating surface for the alternative pathway in sera containing low concentrations of antibodies. However, in the presence of a critical concentration of antibody with specificity for the sialic acid-containing immunodeterminant, alternative pathway activation occurs (Edwards et al, 1980). Several other microorganisms, for example, E. coli capsular type K1 and Neisseria meningitidis groups B and C (Kasper et al, 1973) have capsular polysaccharides that contain terminal sialic acid residues. E. coli K1 exhibit decreased opsonization mediated by the alternative pathway (Van Dijk et al, 1979) and enhanced resistance to phagocytosis (Bortolussi et al, 1979) when compared to E. coli strains without sialic acid in the capsular antigen.

A study of variations in the opsonic recruitment of type III in chemiluminescence (Hastings and Easmon, 1981), described the opsonization to be antibody and complement dependent, but reported variations in the utilization of the classical and alternative pathways. Baker and coworkers (1982) demonstrated that opsonophagocytosis of clinical type Ia isolates by human sera is mediated by the classical pathway in an immunoglobulin-independent manner, but by the alternative pathway in the presence of reasonable levels of specific antibody. It might be concluded that the opsonization of GBS strains is a complex process in which the individual strain and its possible phenotypic variation, together with the antibody specificity and concentration and the reaction of the organism with complement, must be considered.

Human immune response to bacterial carbohydrate antigens

There are many examples of efficient vaccines based on bacterial carbohydrate antigens. Immunization with purified capsular pneumococcal polysaccharide has long been known to protect against systemic infections with homologous strains (MacLeod et al, 1945). Administration of the N. meningitidis group A or C polysaccharide to adults induces the formation of antibodies of the three major classes of immunoglobulins with both bactericidal (Gotschlich et al, 1969) and opsonic properties (Roberts, 1970). Serum antibodies to capsular polysaccharide from Haemophilus influenzae type b confer immunity to invasive diseases with this organism (Anderson et al, 1972; Mäkelä et al, 1977; Peltola et al, 1977).

Although most polysaccharide vaccines have been shown to be immunogenic in adults, they are relatively poor immunogens in infants (Baker et al, 1978). The H. influenzae type b polysaccharide induced a protective immune response only in children older than 18-24 months (Mäkelä et al, 1977; Peltola et al, 1977). This age-related immunological behavior is similar to that observed with other purified bacterial capsular polysaccha-

rides, i.e. for pneumococci and N. meningitidis group A and C (Borogon et al, 1978; Gold et al, 1977) and has been characterized as "thymic-independent" (Parrott and de Sousa, 1971; Robbins et al, 1973). Booster injections of pneumococcal polysaccharide, N. meningitidis group C polysaccharide or H. influenzae type b polysaccharide were reported not to further increase antibody titers in humans of any age (Anderson et al, 1972; Artenstein et al, 1970; Heidelberger et al, 1946; Peltola et al, 1977).

In contrast to the poor immunogenicity of the purified H. influenzae type b polysaccharide in mice and rats, protein conjugates with H. influenzae type b polysaccharide induced the production of considerable amounts of homologous serum antibodies (Schneerson et al, 1980). The antibody response in mice was related to the dose of conjugate, increased with the number of doses and could be enhanced by previous injection of the carrier protein. It was proposed that the H. influenzae type b polysaccharide component of the polysaccharide-protein conjugate is converted to a "thymic-dependent" antigen. These protein-conjugates have also been used for other bacterial polysaccharide antigens, for example for purified pneumococcal polysaccharides. Protein conjugates with the latter induced the formation of high levels of antibodies in rabbits, antibodies which were protective against challenge with the pneumococcus (Goebel, 1939, 1940). Furthermore, E. coli K1- and K13-protein conjugates induced the formation of antibodies to anticapsular polysaccharide in rats and rabbits (Schneerson et al, 1980). Carbohydrate-protein conjugates with Salmonella typhimurium and Klebsiella pneumoniae type K2 have also been used as immunogens and these products enhanced the immunogenicity of the carbohydrate moiety (Geyer et al, 1979; Lindberg et al, 1974; Svenson et al, 1979).

The molecular size is important for the immunogenicity of the polysaccharide. Immunized subjects responded poorly to a preparation of N. meningitidis group A polysaccharide, which was of low molecular weight (Gotschlich et al, 1972). Of two GBS type III polysaccharide vaccines, the polysaccharide with the greater molecular size was significantly more immunogenic (Baker et al, 1978).

The preimmunization antibody level has a considerable influence on the immune response to both a specific (native GBS type III) and a structurally similar bacterial polysaccharide from Pneumococcus type 14 (Baker et al, 1980). Subjects with low levels of antibody to the GBS type III polysaccharide antigen were immunized with a multivalent pneumococcal vaccine and after 4 weeks there was no significant increase in antibody to this antigen. However, subjects with moderate or high preimmunization levels to GBS type III, immunized with the same polyvalent pneumococcal vaccine or native GBS type III polysaccharide, showed significant increases in antibodies. An immunochemically

distinct polysaccharide (Pneumococcus type 14) induced the formation of a population of antibodies in persons who had been sensitized to the specific antigen (GBS type III), but failed to induce antibodies in persons who had not been sensitized. On the other hand, the antibody response to immunization with capsular antigen of H. influenzae type b is independent of preimmunization level (Anderson et al, 1972). The same applies to vaccination with N. meningitidis group A and C polysaccharides (Gotschlich et al, 1972).

It is believed that human antibodies to bacterial polysaccharides consist primarily of IgG and are largely restricted to the IgG2 subclass (Riesen et al, 1976; Yount et al, 1968). A direct correlation between IgG2 concentrations and the antibody response to bacterial polysaccharide antigens has been described (Siber et al, 1980). The level of IgG2 before immunization correlated directly with the mean antibody response to 11 pneumococcal antigens and to the H. influenzae type b antigen. The serum concentrations of IgG2 appear to predict the ability of the individual to produce antibodies to polysaccharide antigens, but not to viral protein antigens. Recurrent infections in an individual with low IgG2 levels may indicate a reduced capacity of the individual to form specific antibody to bacterial polysaccharide antigens. Oxelius (1974) reported on a family with recurrent bronchopulmonary infections most frequently caused by H. influenzae. None of the three affected members had detectable IgG2 levels. Such patients will also exhibit poor antibody responses to polysaccharide vaccines (Siber et al, 1980). The hypothesis has been brought forward that a regulating mechanism, specific as to immunoglobuline subclass, is activated by bacterial carbohydrate antigens (Perlmutter et al, 1978). Such a mechanism could explain the peculiarities that distinguish antibody response to polysaccharides from the response to protein antigens, including poor response in infancy, prolonged high antibody levels after immunization and the apparent absence of secondary responses to booster immunizations (Siber et al, 1980).

Association between antibody response and genetical markers on immunoglobulins

There are several indications of an association between certain allotype markers on immunoglobulins and the antibody response to bacterial antigens. In one investigation, the Gm, A2m and Inv allotypes were examined in adults immunized with H. influenzae type b polysaccharide, N. meningitidis group A and C and pneumococcal polysaccharides (Ambrosini et al, 1982). Post immunization antibody concentrations were significantly higher in G2m(23) patients for H. influenzae type b polysaccharide and for 8 of 11 pneumococcal types. Inv(1) individuals tended to form lower postimmunization antibody concentrations to polysaccharide antigens from H. influenzae type b and N.

meningitidis group A and C than Inv(-1) subjects. The investigators concluded that the G2m(23) allotype, a genetic marker for the IgG2 subclass, identified individuals with enhanced antibody responses to several bacterial carbohydrate polysaccharides.

A highly significant association was found between the Inv(1) allotype and the immune response to H. influenzae and N. meningitidis group C polysaccharide in white children (Pandey et al, 1979). They suggested that associations between specific antibody response and Gm and/or Inv allotypes could be a selective force for the maintenance of the various haplotypes and their frequencies (Steinberg, 1969). However, the selection could also operate through the Ir genes with Gm and Inv antigens serving merely as markers (Pandey et al, 1979). Furthermore, the phenotype Gm -(1,21) was associated with an augmented immune response to the flagellin antigen of *Salmonella adelaide* and Gm (1,21) with a damped response and increased mortality (Mackey et al, 1975; Wells et al, 1971). The low responders, Gm (-1, -21) phenotypes, were assumed to have either a lower immune responsiveness to certain bacterial antigens or a greater predisposition to certain of the illnesses represented in the subjects studied (Mackey et al, 1975).

Animal models for studies of GBS infection

Several animal models suitable for studying GBS infection have been described since Lancefield and coworkers in 1975 described a mouse model for protection tests with GBS types Ia, Ib, Ic and II. It is notable, however, that Lancefield's first experimental studies of GBS in mouse protection models were done already in 1934 and 1935. As mentioned above, Lancefield found that specific antibodies to either polysaccharide or protein antigens can protect against infection with streptococci containing these antigens. She did not, however, perform complete mouse protection studies with GBS type III (Lancefield et al, 1975). The lack of virulence of GBS type III in mice was also reported by others during the following years (Tieffenberg et al, 1978; Wennerström and Shuit, 1978). In most of these studies a GBS type III reference strain, D136C, was used and although several mouse-passages were performed this did not result in a selection of bacteria, significantly virulent for mice. Hence, a chick embryo test (Tieffenberg et al, 1978) was the first animal model developed for the study of infections with and immunity to GBS type III. A clinical isolate of type III and a type III reference strain could induce septicemia in chick embryos and type-specific rabbit antisera to protect the embryos. A good correlation between IgG antibody titers, measured by indirect immunofluorescence in human sera, and passive protection in chick embryos has been described for types Ia, Ib, Ic, II and III (Vogel et al, 1979).

The first successful mouse protection model with type III was described in 1979 by Baltimore and coworkers, who found 6 clinical isolates but not the reference strain D136C to be virulent for mice when injected intraperitoneally. Hyperimmune rabbit antisera to a clinical isolate of type III was protective.

Several animal models for studies of type Ia have been described (Shuit and DeBiaso, 1980; Stewardson-Krieger et al, 1977; Wennerström and Shuit, 1978). Stewardson-Krieger and coworkers demonstrated a protective effect of human sera in a mouse model for type Ia; the protective antibodies were most frequently of the IgG class, but in one serum of IgM class (1977). Shuit and DeBiaso (1980) developed a rat model in which the kinetics of the phagocyte response to intraperitoneal infections with type Ia could be studied in baby and adult rats. The neutrophil response to type Ia infection was found inadequate and could contribute to the high mortality associated with this infection. Finally, a rat model for studies of vaginal colonization and perinatal acquisition of types Ia, II and III has been developed (Ancona and Ferrieri, 1979). It may shed light on the dynamics of vaginal carriage and mother-to-infant transmission of GBS.

Mucin has been widely used to enhance intraperitoneal infections in mice. A mouse mucin model for GBS in which the bacteria were suspended in sterile hog mucin (Flemming, 1980) showed enhancement of virulence of a type III reference strain. Another way to enhance the virulence of type III in mice, is to inject oleic acid intraperitoneally together with the bacteria (Kretschmer et al, 1980). This suggests that the peritoneal macrophages play a role in murine defence against intraperitoneal infections with type III.

Group B streptococcal infection in the neonatal period

GBS is the most common cause of septicemia and meningitis in the neonatal period in the industrialized part of the world (Baker, 1977; Eickhoff et al, 1964; Franciosi et al, 1973) but also occur in developing countries (Jamal et al, 1982). The incidence of neonatal GBS infection has increased during the last two decades (Baker, 1980). The number of newborns contracting GBS septicemia and/or meningitis has been estimated to 12.000-15.000 per year in the United States (Baker, 1977). The mortality rate is 30-44 % (Anthony and Okada, 1977; Baker, 1980). The attack rate for the early onset infection varies from 1.3 to 3.0/1000 live births and for the late onset infection it is estimated to be 0.6 to 1.0/1000 live births in the U.S. (Baker, 1980). In Sweden there are at least 110 cases of neonatal GBS infection every year (Christensen et al, 1982a, b). The male-female ratio is equal in both early and late onset GBS disease and there appears to be no association with race or socioeconomic status (Baker, 1980).

A major reservoir for GBS in the human is the female genital tract (Christensen, 1980; Eickhoff et al, 1964; Fry, 1938; Hood et al, 1961). The two forms of neonatal GBS infection - early onset and late onset infection - are probably acquired in different ways. In the early onset form the GBS are probably transmitted from mother to child, either in utero or during passage through the birth canal. This is indicated by the early symptoms, the prevalence of GBS in the birth canal, the post-partum isolation of GBS from mothers of septic infants and the identity of maternal and infant serotypes (Bergqvist et al, 1971; Bevanger, 1974; Hey et al, 1973; Franciosi et al, 1973). It has been proposed that the late onset form results from nosocomial spread of GBS in maternity units (Aber et al, 1976; Steere et al, 1975).

The GBS carrier rate among pregnant women varies widely, from 5 to 30 % (Aber et al, 1976; Anthony and Okada, 1977; Baker and Barrett, 1973; Christensen et al, 1981b; Embil et al, 1978; Ferrieri et al, 1977; Franciosi et al, 1973). Vertical transmission rates of 50-72 % among the neonates born to women who are urogenital carriers of GBS have been reported (Aber et al, 1976; Anthony and Okada, 1977; Baker and Barrett, 1973; Christensen et al, 1981b). Infants of mothers who are solely urethral carriers are contaminated to a lesser extent than infants of combined urethral/cervical carriers (Christensen et al, 1981b). A small number of infants to culture-negative women are colonized with GBS during the first days of life, indicating a nosocomial spread in maternity units (Aber et al, 1976; Anthony et al, 1976; Christensen et al, 1981b; Easmon et al, 1981; Paredes et al, 1977; Steere et al, 1975). In the early onset form, type III dominate (80-90%) in some reports (Baker and Barrett, 1974; Wilkinson, 1978c) while others found a distribution closely reflecting the distribution among parturients (Christensen et al, 1981b; Franciosi et al, 1973; Paredes et al, 1977; Wilkinson, 1978a, b). In the late onset form, 93 % of the strains isolated are of type III (Baker and Barrett, 1974).

In the early onset form the neonates fall ill within 10 days of birth, usually within 48 hours. The late onset form occurs after the age of 10 days (Baker and Barrett, 1973; Franciosi et al, 1973). In children older than 3 months GBS are rarely associated with serious infections (Anthony and Okada, 1977). The early onset form affects preterm as well as fullterm infants, infants whose mothers have had obstetric complications and those without complications (Baker, 1980). The first signs of illness are often abnormal respiratory patterns with tachypnoe, cyanotic spells or apnoeic episodes. In some newborns the disease is rapidly fatal with death occurring within 1 hour after onset. Approximately 30 % of the infants with septicemia have concomitant meningitis and 40 % have pulmonary involvement (Anthony and Okada, 1977). The clinical and radiological differentiation

between streptococcal pulmonary disease, respiratory distress syndrome (IRDS) and pulmonary maladaptation syndrome (PMA) is often difficult (Anthony and Okada, 1977). To distinguish GBS pneumonia from the others, the isolation of the organism is necessary (Christensen et al, 1982a; Christensen et al, submitted). The overall mortality rate of GBS septicemia in newborns is 44 % (Anthony and Okada, 1977). It is significantly higher in the early onset infection (55 %) than in the late onset form (23 %). Early onset infection and pneumonia without septicemia in the infant can be associated with fever and other signs of postpartum infections in the mother (Bergqvist et al, 1971; Bobitt and Ledger, 1976; Christensen et al, 1982a; Eickhof et al, 1964).

The late onset form of GBS neonatal infection may occur from 10 days to several weeks after birth and meningitis is the most common manifestation while pneumonia is rare. The late onset form is infrequently associated with obstetrical complications (Anthony and Okada, 1977). After late onset meningitis neurological sequelae are found in one third of the surviving infants (Horn et al, 1974).

The histological findings in fetal GBS disease are often few. The appearance of cocci inside the hyaline membranes seems to be typical of GBS disease and does not occur in other forms of septicemia (Ablow et al, 1976; Katzenstein et al, 1976). An inflammatory reaction may be completely absent even after several hours of infection (Christensen et al, 1982a).

Infants born to urogenital GBS carriers contract pneumonia more frequently than infants born to non-carriers (Christensen et al, 1982a). GBS are also the most common bacterial cause of neonatal pneumonia without septicemia. In a recent study 8 of 14 infants with pneumonia harboured GBS as the only pathogenic bacteria in contrast to 4 of 24 infants with pulmonary maladaptation syndrome (Christensen et al, 1982a). The incidence of pneumonia caused by GBS without septicemia is estimated to 1 per 25 parturient urogenital GBS-carriers. It is also notable that an increased risk of GBS disease in twins has been reported (Edwards et al, 1981; Pass et al, 1980).

Pathological fetal heart rate (FHR) findings during labour, pH values below 7.20 during the first stage of labour and postmaturity have been found significantly more often among infants to urogenital carriers of GBS than among infants to non-carriers (Christensen et al, 1980b). Other sites and manifestations of streptococcal infection in the newborn are remarkably varied. Haemorrhagic phenomena, pyartrosis, conjunctivitis, empyema, endocarditis, ethmoiditis, endophthalmitis, omphalitis, osteomyelitis, otitis media, pericarditis, peritonitis, subdural effusions, cutaneous lesions have been reported (reviewed by Anthony and Okada, 1977; Baker, 1980).

In the mothers GBS cause postpartum endometritis in 5 % of all GBS carriers and sometimes septicemia, especially following cesarean section (Christensen et al, 1982a). Pass and coworkers (1982) reported the incidence of GBS puerperal septicemia as 2/1000 deliveries in a population with a known high incidence of GBS carriage. Faro (1981) found GBS as a major cause of puerperal infection in 40/3100 deliveries. GBS bacteraemia occurred in 10 of these patients which gives a rate of 3.2/1000 deliveries.

Treatment of GBS infection in the neonatal period

Penicillin G is clearly the preferred drug for the treatment of group B streptococcal disease in infants and adults (Anthony and Concepcion, 1975; Eickhoff et al, 1964; McCracken and Feldman, 1976). It is essential to keep the benzylpenicillin dosage high in order to reach bactericidal concentrations in the CSF. McCracken and Feldman (1976) suggested a dosage of 150 mg/kg/day, divided in 2-4 doses depending on age, rather than the commonly recommended 100 mg/kg/day. A combination of penicillin and aminoglycoside is often used because of the synergistic effect demonstrated in vitro (Baker CN et al, 1981). As soon as the CSF is sterile, the aminoglycoside therapy can be discontinued, whereas benzylpenicillin should be continued for a total of 2 weeks (Baker, 1980; Christensen et al, 1982a). In the future it is likely that some of the new cephalosporines will be tried, because of the high sensitivity of GBS to some of these drugs (Baker CN et al, 1981; Bayer et al, 1982).

Transfusion of neonates with fresh whole blood containing GBS antibodies has been shown to increase the serum opsonic activity and may together with conventional therapy increase the survival in neonatal GBS (Shigeoka et al, 1978b). Antibiotic therapy may eradicate or suppress group B streptococci in the birth canal, but the effect is temporary and the streptococci most often reappear (Anthony and Okada, 1977). Data on eradication with antibiotics of GBS from superficial body sites in the newborns are more limited but suggest that penicillin, even when given intravenously does not consistently eliminate GBS from the throat, umbilicus or rectum (Paredes et al, 1976; Steere et al, 1975). Prophylaxis with single dose penicillin against neonatal GBS infections has been studied (Siegel et al, 1980). A significant decrease in the incidence of disease caused by penicillin susceptible organisms was found, while there was an increase of infections caused by penicillin resistant pathogens. However, late intrauterine GBS infections may also occur and these infections are not preventable by the administration of penicillin after birth.

Aims of the Investigation

- to compare serum levels of type-specific antibodies in pregnant GBS carriers and non-carriers;
- to elucidate the antibody response to bacterial carbohydrate antigens in mothers of GBS-infected infants;
- to investigate the distribution of Gm allotypes in mothers of GBS infected infants;
- to investigate the role of R-protein in experimental mouse infections and in human GBS colonization and infection;
- to determine the serum levels of antibodies to R-protein in mothers of GBS infected infants;
- to determine levels of type-specific antibodies in commercial IgG preparations;
- to elucidate GBS urogenital carriage and antibody response in pregnancy following loss of fetus in GBS infection.

Present Investigations

A. MATERIALS AND METHODS

Groups of individuals studied.

The patient groups and controls studied in papers I, II, III, V, VI and VIII are shown in Table 1. Data concerning GBS neonatal infection and mothers of GBS infected infants in papers II, III, VI and VIII are summarized in Tables 2 and 3, respectively. All serum samples and bacteriological specimens were collected during 1977-81 from various parts of Sweden.

Quantitation of type-specific GBS antibodies using radiolabelled protein A

Quantitations of type-specific IgG antibodies to GBS types Ia, Ib, II and III (I, II, III, VI, VII and VIII) were performed by the use of ^{125}I radiolabelled protein A of Staphylococcus aureus. The sera were absorbed, to remove group specific antibodies and then mixed with a standardized suspension of the GBS type against which antibodies were to be measured. After washings, radiolabelled protein A was added and the bacteria were again washed. The antibody levels were expressed as the radioactivity detected in the bacteria pellets, expressed in counts per minute (cpm).

The protein A method preferentially detects antibodies of the IgG subclasses 1, 2 and 4. Studies with rabbit antisera against various types of GBS have demonstrated that the procedure detects type-specific antibodies (Christensen et al, 1980a). One advantage of the method is that the native type antigens on the surface of the bacteria are used for measurement of antibodies, an important point in the detection of "protective" antibodies especially against GBS type III. It has been shown that whole bacteria detect opsonic antibodies to a greater extent than preparations of antigens (Rote et al, 1980).

Table 1. Groups of individuals studied in papers I, II, III, VI and VIII.

Paper no	Group (C=controls P=patients)	Criteria for selection of the individuals	Group totals (n)	Distribution of serotypes of GBS isolated from the urogenital tract/infant (blood, CSF)			Age range of the mothers (mean) years
				Ia	Ib	II III	
I	P	healthy urogenital carriers giving birth to healthy infants	53	20/ni*	0/ni	10/in 23/ni	20-39 (28.5)
C		healthy non-carriers giving birth to healthy infants	20				20-39 (28.6)
II	P	mothers of GBS-infected infants	16	2/2	4/4	2/2 8/8	20-36 (27.3)
C		urogenital GBS carriers giving birth to healthy infants	29	10/ni	5/ni	5/ni 9/ni	20-36 (26.6)
III	P	mothers of GBS-infected infants	34	7/7	4/4	8/8 13/13**	20-39 (28.6)
VI	P	mothers of GBS infected infants	17		7/7	10/10	20-39 (29.4)
C		urogenital GBS carriers giving birth to healthy infants	26			13/ni 13/ni	20-39 (30.0)
VIII	P	mothers of GBS-infected infants	22	4/4	4/4	3/3 11/11	20-36 (29.0)
C		urogenital GBS carriers giving birth to healthy infants	40	10/ni	10/ni	10/ni 10/ni	20-36 (28.2)

*ni = not investigated

** 2 strains not typed.

Table 3. Data concerning the mothers of GBS infected infants taking part in papers II, III, VI and VIII.

Patient no.	Included in paper no.	GBS type isolated from infant (blood/CSF) and mother (urogenital tract)	Infection: Early (E) or late (L) onset GBS disease, or intrauterine death (IU)	Outcome: infant died (D) or survived (S)	Maternal antibody level against the infecting type high (H) or low (L)
1	II, III, V	Ia	E	S	L
2	III, V, VIII	Ia	E	D	L
3	III, V, VI, VIII	Ia (II)*	E	D	L
4	III, V, VIII	Ia	E	S	L
5	III, V, VIII	Ia	E	S	L
6	III, V	Ia	E	D	L
7	III, V	Ia	E	S	H
8	II, V	Ia	E	D	L
9	II, III, V, VIII	Ib	E	S	L
10	II, III, V, VIII	Ib	E	S	L
11	II, V	Ib	L	S	L
12	II, III, V, VIII	Ib	E	S	H
13	V, VIII	Ib	E	S	L
14	III, V	Ib	E	S	H
15	III, V, VI, VIII	II	E	D	L
16	II, III, V, VI, VIII	II	E	D	L
17	III, V, VI	II	E	S	L
18	III, V, VI	II	E	S	L
19	III, V, VI	II	E	D	L
20	III, V, VI	II	E	S	L
21	III, V	II	L	S	L
22	II, III, VIII	II (III)*	IU	-	L
23	II, III, V	III	L	S	L
24	II, III, V	III	L	S	L
25	II, V, VIII	III	E	S	H
26	III, V, VI, VIII	III	E	S	L
27	III, VIII	III	IU	-	L
28	III, V, VI, VIII	III	E	S	L
29	II, III, V, VI, VIII	III	E	S	L
30	III, V, VIII	III	E	S	L
31	II, III, V, VI, VIII	III	E	S	L
32	II, III, V, VI, VIII	III	E	S	L
33	III, V, VI, VIII	III	E	D	L
34	III, V, VIII	III	E	D	L
35	III, V, VI	III	E	S	L
36	III, V, VIII	III	E	S	H
37	II	III	E	D	L
38	II	III	E	D	L
39	V, VI	III	IU	-	L
40	V, VI	III	E	D	L
41	V, VI	III	E	S	L
42	III	**	E	S	L
43	III	**	E	S	L

*= the type in parentheses was isolated once from the urogenital tract

**= unknown type

***= nd = not done

no of bacterial antigens, to which the mater- nal antibody le- vel was below a selected limit (see II) (9 antigens tested)	Gm type 1 5		Presence of R-protein on the surface of the isola- ted strain	Maternal antibody level against R-protein L = low, H = high	Patient success- ful preg- nant again following the preven- tion pro- gram	Remarks
4	+	+	R-	nd		
nd***	-	+	R-	nd	yes (Lund)	
nd	+	+	R-	H		
nd	+	-	R-	nd		
nd	-	+	R-	nd	yes (Stockholm-Japan)	
nd	+	+	R-	nd	yes (Stockholm)	
nd	+	+	R-	nd		
0	nd	nd	R-	nd		
4	+	-	R-	nd		
4	-	+	R-	nd		
0	nd	nd	R-	nd		
0	-	+	R-	nd		juvenile di-
nd	nd	nd	R-	nd		betes melli
nd	+	+	R-	nd		
nd	-	+	R-	L		
3	-	+	R-	H	yes (Lund)	
nd	-	+	R+	L	yes (Stockholm)	
nd	-	+	R+	L		
nd	+	+	R+	L	yes (Stockholm)	
nd	-	+	R+	L		
nd	-	+	R+	nd	yes (Stockholm)	
4	-	+	nd	nd	yes (Lund)	
4	-	+	R+	nd		
0	-	+	R-	nd		
0	anti-Ig		R+	nd		ABO-immuniza-
nd	+	+	R+	L		tion
nd	+	-	nd	nd		
nd	-	+	R-	H		
3	+	-	R+	L		
nd	-	+	R+	nd		
4	-	+	R+	L		
8	-	+	R+	L		
nd	+	-	R+	L	yes (Stockholm)	
nd	-	+	R+	nd	yes (Västervik)	
nd	-	+	R-	L		
nd	-	+	R+	nd		umbilical
0	nd	nd	nd	nd		catheter
0	nd	nd	nd	nd		
nd	nd	nd	R+	L	yes (Lund)	
nd	nd	nd	R+	L		
nd	nd	nd	R+	L		
nd	-	+	nd	nd		
nd	-	+	nd	nd		

Table 2. Data concerning the GBS-infections studied in papers II, III, VI, and VIII.

STUDY PERIOD	No. of patients
The specimens were collected during 1977-81	
GEOGRAPHICAL DISTRIBUTION OF THE PATIENTS	
Lund	12
Stockholm, Karolinska Sjukhuset	11
Stockholm, Södersjukhuset	10
Southern part of Sweden	10
Total No	43
CLINICAL RESULTS	
mothers to GBS-infected infants included in studies II, III, VI, VIII	43
infants with early onset GBS infection mortality rate	36 (84%) 33%
infants with late onset GBS infection mortality rate	4 (9%) 0%
intra-uterine death or late spontaneous abortion	3 (7%)
BACTERIOLOGICAL FINDINGS	
serotype distribution: Ia	8 (19.5%)
Ib	6 (15%)
II	8 (19,5%)
III	19 (46%)
unknown	2

B. IMMUNOLOGICAL RESPONSE TO BACTERIAL CARBOHYDRATE ANTIGENS (I-III)

Type-specific serum antibodies against GBS among pregnant women (I)

Previous studies have indicated that the production of GBS antibodies can be stimulated by urogenital carriage of GBS. Female patients attending a venerological outclinic were found to have higher levels of type-specific antibodies than patients attending a gynecological out-clinic and girls 10-12 years of age. This could be correlated to the higher rate of GBS carriage in the urogenital tract in the first mentioned group (Christensen et al, 1974; Christensen et al, 1981a). As already mentioned mothers of GBS-infected infants are deficient in antibodies against the infecting GBS type (Baker and Kasper, 1976a; Christensen et al, 1980a; Hemming et al, 1976; Stewardson-Krieger et al, 1977; Vogel et al, 1979). Nevertheless, these mothers are urogenital carriers of GBS. It was therefore of interest to study GBS antibody levels in parturient urogenital GBS carriers in order to investigate whether the presence of the organisms in the urogenital tract regularly leads to production of specific serum antibodies.

In paper I, sera from 53 parturients who were urogenital carriers of GBS (study group) and sera from 20 culture negative parturients (control group) were investigated for antibodies to types Ia, Ib, II and III of GBS; all women gave birth to healthy infants.

The GBS carriers were found to have significantly higher serum levels of antibodies to types Ia and II than the non-carriers ($p < 0.05$) (I, fig 1). The levels of antibody to type Ia were found to be significantly higher among carriers of type Ia and type III than among carriers of type II and the controls ($p < 0.05$). The serum levels of type-specific antibodies could not be related to the type of GBS isolated from the urogenital tract, except for type Ia carriers who had higher levels of type Ia antibodies than the controls (I, fig 2). Vogel and coworkers (1980) using an immunofluorescence method for quantitation of antibodies described similar results. No differences in serum levels of type-specific antibodies against GBS were found between primigravidae and multigravidae, either in the study group or in the control group. GBS carriers aged 20-29 years (younger group) had higher levels of antibody against types Ib and II than the older carriers, aged 30-39 years ($p < 0.05$) (I, fig. 3). The younger carriers also showed higher serum antibody levels against types Ia and II than did the younger controls

($p < 0.05$). No difference was found in antibody levels to any type of GBS among the two age groups in the controls. In the younger age group, carriers of type Ia had higher antibody levels to the types Ia and II than the type II carriers ($p < 0.05$) (I, fig. 4). Furthermore, the younger type III carriers had higher levels of antibody to type Ia compared to carriers of type II and the controls and also higher levels of type II antibodies than the controls ($p < 0.05$). In summary, among the GBS-carriers, the levels of antibodies were generally higher in younger than in older parturients, whereas no such difference was shown among the controls. Similar results have been obtained with Chlamydia trachomatis (Mårdh et al, 1980).

It was concluded from the results in paper I, that stimulation to production of type-specific serum antibodies against GBS in man occurs through urogenital colonization. Pregnant GBS carriers had generally higher levels of type-specific antibodies to GBS than non-carriers, irrespective of the type of GBS harboured in the urogenital tract. As will appear from the following, mothers of GBS-infected infants are in general exceptions to this rule (II, III, VIII) although they may be urogenital carriers for years (VIII).

Antibody response to bacterial carbohydrate antigens in mothers of GBS infected infants (II).

Several studies have shown that mothers of GBS infected infants are deficient in type-specific IgG serum antibodies against the strain infecting their infant (Baker and Kasper, 1976a; Christensen et al, 1980a, VIII). There may be more than one mechanism responsible for the low antibody levels against GBS. One explanation might be that mothers of GBS-infected infants were colonized relatively close to the delivery, and that protective antibodies therefore had not had time to develop. Another possibility would be that these parturients were incapable to respond to GBS antigens in particular or to bacterial carbohydrate antigens in general. In paper II the serum levels of antibodies against a variety of bacterial carbohydrate antigens in mothers of GBS infected infants were studied.

Sera from 16 mothers of infants (or fetuses) with serious GBS infection were investigated (study group). The control group consisted of 29 parturients who were urogenital GBS carriers at the delivery, but gave birth to healthy infants.

As expected from a previous study (Christensen et al, 1980a) mothers belonging to the study group showed low IgG levels against the infecting GBS type (II, fig. 2) However, it was also found that the study group, when compared to the control group (II, fig. 1), had lower levels of IgG antibodies against all types of GBS, irrespective of which type had infected the

infant.

IgG and IgM antibodies to Salmonella typhimurium, Salmonella enteritidis, Yersinia enterocolitica type 03, Francisella tularensis lipopolysaccharides as well as IgG antibodies to Streptococcus pneumoniae types 3, 6, 9, 19 and 23 capsular polysaccharides were detected with a microplate ELISA technique (Karlsson et al, submitted).

For all nine antigens studied the geometric means of IgG concentrations were lower in the study group than in the control group, while IgM concentrations were higher against the four antigens tested in the control group. In order to avoid that the odd low IgG or high IgM levels influenced the mean values, limits of antibody concentrations were selected and levels above or below the limits were registered among the patients and controls (II, table 1). Using this method, significantly more individuals in the study group than among the controls had IgG antibodies below the selected limits for 8 of 9 carbohydrate antigens (table 4). In all, 9 of 16 in the study group and 2 of 29 controls had IgG antibody levels below the selected limits, and each of these individuals had low levels against at least 3 antigens. On the other hand, there were significantly ($p < 0.01$) more individuals in the study group with high IgM levels against the Salmonella enteritidis antigen (Table 4).

The medical records of the mothers of GBS infected infants did not reveal an abnormally high number of infections with bacteria carrying carbohydrate antigens in the past. Two plausible explanations for this comes to mind: firstly - IgG antibodies against the antigens studied were not completely absent; secondly - the mothers might compensate their relative IgG anti-carbohydrate deficiency through the production of IgM antibodies. Since IgG, but not IgM, passes the placenta their infants, but not they themselves, would be susceptible to e.g. GBS infections. Recently, Anthony and coworkers (1982) reported that measurable IgM antibody to type III was present in 41/50 (82%) pregnant GBS type III carriers. They suggested that if IgM is protective, it may account in part for the infrequency of invasive infection outside the newborn period.

In conclusion, a majority of mothers to GBS infected infants were found to have low levels of IgG antibodies to carbohydrate antigens in general indicating that these mothers were poor IgG responders to bacterial carbohydrate antigens.

Table 4. Serum levels of IgG and IgM antibodies against bacterial carbohydrate antigens in mothers of GBS infected infants compared with controls.

Bacterial antigen	No. of individuals within the selected limits given in publication II.		p-value χ^2 test
	study group n = 16	controls n = 29	

IgG antibody

<u>Salmonella</u> BO	3	0	0.037
<u>Salmonella</u> DO	3	0	0.037
<u>Y. enterocolitica</u> 03	5	0	0.004
<u>F. tularensis</u>	4	0	0.012
<u>S. pneumoniae</u> type 3	-	-	-
<u>S. pneumoniae</u> type 6	5	1	0.015
<u>S. pneumoniae</u> type 9	6	2	0.015
<u>S. pneumoniae</u> type 19	6	2	0.015
<u>S. pneumoniae</u> type 23	6	2	0.015

IgM antibody

<u>Salmonella</u> BO	-	-	-
<u>Salmonella</u> DO	5	1	0.015
<u>Y. enterocolitica</u> 03	-	-	-
<u>F. tularensis</u>	-	-	-

Maternal Gm allotype and neonatal GBS infection (III)

It appeared from the investigations reported in paper II, that mothers of GBS infected infants were poor IgG-responders to bacterial carbohydrate antigens; the question arose whether this deficiency was genetically determined. In this respect, it was of interest to study Gm-allotypes in mothers of GBS infected infants. Several investigations indicate an association between Gm allotypes and immuneresponse to certain antigens (Madkey et al, 1975; Nevo, 1974; Pandey et al, 1979; Rivat et al, 1978; Wells et al, 1971) as well as between Gm allotypes and the general ability to survive infectious diseases (Schanfield, 1975; deVries et al, 1979).

Sera from 34 Swedish mothers of infants having contracted GBS neonatal septicemia and/or meningitis or intrauterine death caused by GBS, were investigated. Thirtytwo of these women were urogenital carriers of the GBS type infecting their infant.

Twentyeight of the mothers showed very low levels of antibodies to the GBS type infecting their infant, as found in papers II, VI and VIII.

Gm -typing for G1m(1) and G3m(5) was performed according to standard methods (Grubb, 1970).

The frequencies of types Gm (1,-5), Gm(1,5) and Gm (-1, 5) among the 34 mothers were compared to the frequencies expected in the Swedish population. The distribution of Gm types in the study group differed significantly ($p < 0.01$) from the distribution in the Swedish population (Table 5).

The abnormal distribution of Gm allotypes in mothers of GBS infected infants fits well with the assumption that the IgG immuneresponse to bacterial carbohydrate antigens is influenced by genes closely associated with the Gm loci, which are tightly linked to the various subclasses of IgG (Grubb, 1970).

Furthermore, this finding indicates that these mothers are poor IgG responders to bacterial carbohydrate antigens (II) on a genetic basis. It has been convincingly shown that in mice the immune responsiveness to dextran and to pneumococcal polysaccharide type 14, respectively, is controlled by loci closely linked to Ig allotype loci (Blomberg et al, 1972; Mäkelä et al, 1980).

Table 5. The distribution of Gm phenotypes in mothers of GBS infected infants.

Phenotype	observed* no.	expected* no.
Gm(1,-5)	5	4.15
Gm(1,5)	7	15.46
Gm(-1,5)	22	14.40

* The observed frequencies differed significantly from the frequencies expected from studies of the Swedish population ($p < 0.01$).

C. EPIDEMIOLOGICAL AND IMMUNOLOGICAL ASPECTS OF GBS R-PROTEIN (IV-VI)

In the previously reported studies (II, III), it was documented that mothers of infants with GBS septicemia seem to be poor IgG responders to bacterial carbohydrate antigens in general. It was not known whether the IgG response to bacterial protein antigens might also be defective. We therefore decided to study the R-protein of GBS. Despite the fact that the R-protein was discovered over 30 years ago, little was known about the distribution of this antigen in GBS from human specimens or about the importance of this antigen for the virulence of the bacteria. It was therefore necessary to study the epidemiological and virulence aspects of R-protein before studying the human antibody response to the antigen.

Protective effect of R-protein antibodies in GBS type II (IV)

R-antigen has been used as an epidemiological marker in bovine GBS strains (Pattison et al, 1955 a,& b). The antigen is closely related to the R-protein found in group A streptococci of type 28 (Lancefield and Perlman, 1952), and has also been found in groups C, G and L (Maxted, 1948; Perch and Olsen, 1964). The R-protein was for many years considered to be without importance for the virulence of GBS, a view based on the observation that R-protein antibodies were not protective in mice against infections with group A streptococci type 28 (Lancefield and Perlmann, 1952). In consequence, the R-protein has not been used in the GBS typing scheme.

The studies reported in paper IV were based on the assumption

that antibodies to R-protein might protect mice from infection with GBS carrying this antigen. If so, R-protein also might be important for the virulence of GBS in man. In order to study the mouse-protective effect of R-protein antibodies, the protein was isolated from an alkaline extract of a GBS type III strain by the use of affinity chromatography. Using this R-antigen for immunization of rabbits it was possible to prepare a monospecific anti-R serum without absorption procedures; the latter were avoided since e.g. streptococcal antigens solubilize during the absorption might interfere with the results.

The antiserum to purified R-protein precipitated the R-protein, giving a line of non-identity with the type III antigen/anti-III precipitate (IV, fig. 1). In crossed immunoelectrophoresis the R-protein preparation gave one anodally located precipitate with the antiserum to the clinical type III isolate carrying R-protein as well as with the antiserum to the R-protein (IV, fig. 2). The R-protein antiserum was tested in double diffusion-in-gel against extracts of the reference strains and all clinical isolates. Precipitates were only obtained with strains containing R-protein and in these cases only a single precipitate was seen.

Twelve clinical GBS isolates, 6 type II and 6 type III, were tested for binding of radiolabelled protein A after coating with the monospecific R-protein antiserum for demonstration of R-protein as a surface antigen in GBS types II and III. The R-protein antiserum elicited a binding of 85-90 % for 3 type II and 4 type III strains indicating that the R-protein was carried on the surface of the cells. To the remaining strains less than 7 % protein A was bound, indicating that these isolates were all without R-protein.

The mono-specific R-protein antiserum gave clear-cut protection of mice against three type II isolates containing R-protein but not against three type II isolates without R-protein or against any of six type III isolates, irrespective of the presence of R-protein in these strains (IV, table 2). Furthermore, the results of phagocytosis experiments with mouse macrophages, confirmed that type II strains carrying R-protein were opsonized by R-protein antiserum.

The finding that R-protein antiserum was not protective against infection with type III, R+ strains might have several explanations. Thus, R-proteins might differ with respect to chemical composition between type II and type III. It should be noted, however, that the antiserum protecting against type II, R-negative strains was raised against R-protein prepared from a type III strain. The possibility also exists that the R-protein antiserum reacted with some unknown component closely associated with the R-protein and exerted its protection in this way. Finally, the type III carbohydrate might interfere with the

opsonization of type III strains via anti-R-antibodies.

The most plausible explanation was, however, that R-protein antibodies protected mice against type II strains carrying this antigen. Thus according to classical definitions, GBS type II can be divided into two subgroups, for which we suggest the names II, R+ and II, R-. Furthermore, the results also suggested that R-protein might play a role in GBS type II infections in man. Encouraged by these results we decided to study the distribution of R-protein among human GBS strains (V) and human antibody response against R-protein (VI).

R-protein in clinical isolates of GBS (V)

In order to study the distribution of R-protein among human GBS strains, 241 clinical isolates of GBS were studied for the presence of R-protein, using immunodiffusion, coagglutination and radiolabelled protein A (IV). The results are given in Table 6. R-protein was not detected in any of the GBS type Ia or Ib, occurred in GBS type II, but was most frequently seen in GBS type III. When comparing strains from infected and non-infected individuals there was no difference between the occurrence of R-protein. Paired strains (GBS type II and III) from mother and infant were identical as to the presence or absence of R-protein.

The finding that 87 % of type III strains from the urogenital tract contained R-protein confirmed the frequency of 93 % found by Jensen (1980b), whereas Jelinkova (1977) reported only 16 %. She also reported that 56 % of human isolates of GBS type II carried R-protein, which correlates well with our frequency of 51 %, while Jensen reported 30 %. In strains isolated from GBS infected infants, R-protein was found in 12/14 (86 %) type III strains, corresponding well to the frequency of type III in the total material. It is difficult to assess the clinical importance of R-protein in type III strains. R-protein is generally considered to be "unstable" (Patterson and Hafeez, 1976). The present results demonstrated that R-protein in fact was stable as an epidemiological marker in the sense that concordant results were obtained with paired strains from mother and infant. In the total material of 241 GBS strains studied 46 % carried R-protein. With a urogenital GBS carrier rate of 16 % (Christensen and Christensen, 1978) about 8 % of the fertile female population is constantly exposed to R-protein.

Table 6. Occurrence of R-protein in 241 clinical GBS isolates from human sources.

Strains studied	Total no. of strains	No. of strains with R-protein/No. of strains tested			
		type Ia	type Ib	type II	type III
Total material	241	0/79 (0%)	0/17 (0%)	22/43 (51%)	89/102 (87%)
strains isolated from infants with septicemia/meningitis	30	0/8 (0%)	0/6 (0%)	1/2 (50%)	12/14 (86%)
type II and III strains isolated from infant/mother pairs:					
non-infected infants	25	-	-	6/10 (60%)	14/15 (93%)
infants with septicemia/meningitis	12	-	-	1/2 (50%)	9/10 (93%)

Human immune response to GBS R-protein (VI)

In paper IV we demonstrated that rabbit R-protein antibodies protected mice from infection with GBS type II carrying R-protein. In paper V we found 51 % of GBS type II and 87 % of GBS type III to carry R-protein revealing that R-protein is commonly present in strains involved in human neonatal infections. These findings indicated that R-protein might be suitable for the study of the humoral response to protein antigens in mothers of GBS-infected infants, a question of considerably interest in the light of their poor IgG response to carbohydrate antigens (II).

Sera from 17 mothers of GBS infected infants were investigated for antibodies to R-protein. Of the infecting GBS, 4 isolates of type II and 8 isolates of type III were R-protein positive while 3 and 2 were R-negative, respectively. The control group consisted of 26 parturient urogenital GBS carriers (5 isolates of type II and 10 isolates of type III were R-protein positive while 8 and 3 were R-negative, respectively), who gave birth to healthy infants. IgG antibodies against R-protein were quantitated with the protein A method (Christensen et al, 1980a). The sera were absorbed with a GBS strain type III lacking R-protein and antibodies were quantitated with a type III, R-positive strain.

In mothers of infants infected with R-positive strains of type II the R-protein antibody levels were significantly lower than in the controls, i.e. carriers of R-positive type II strains ($p < 0.05$) (VI, fig. 3). Similar results were obtained with R-positive type III strains ($p < 0.01$). No differences were found in R-protein antibody levels between the study group and controls exposed to R-negative strains of type II and III. Neither was there a difference between mothers of infants infected with II, R-positive and II, R-negative or between types III, R-positive and III, R-negative. However, when all mothers of infants infected with GBS strains carrying R-protein, irrespective of type, were compared to mothers of infants infected with R-negative strains there were significantly higher antibody levels among the R-negative group ($p < 0.05$) (VI, fig. 3).

Thus, it was demonstrated that although mothers of GBS infected infants exhibit a poor IgG response to carbohydrate GBS antigens, this is not always the case for the R-protein antigen (II, III, VIII). However, the antibody levels to R-protein among mothers of infants infected with R-positive GBS were significantly lower than among the controls carrying the same types but giving birth to healthy infants. This corroborates the findings in mice that R-protein antibodies are protective against infection with R-positive type II strains (IV).

In conclusion, this paper demonstrated that mothers of infants infected with R-positive GBS, have low levels of R-protein antibodies. Together with the results of the mouse-protection tests this indicates that R-protein is of significance for the virulence of GBS. However, the low antibody levels to R-protein were not a general characteristic of mothers of GBS-infected infants, a finding of considerable interest to the understanding of the maternal immunological aberrations in severe neonatal GBS-infections.

D. PREVENTION OF NEONATAL GBS DISEASE (VII-VIII)

GBS antibodies in commercial IgG preparations (VII)

Maternal anti-GBS IgG, supplied through placenta, seems to protect against neonatal GBS infections (Baker and Kasper, 1976a; Christensen et al, 1980a; Hemming et al, 1976). The studies reported above (II, III) indicate that mothers of GBS-infected infants might be poor responders to bacterial carbohydrate antigens on a genetic basis. These findings are not encouraging for a successful active immunization against neonatal GBS disease. They indicate that passive immunization of the mothers at risk would be more adequate.

In order to study this possibility, 53 commercial batches of IgG were quantitated for type-specific GBS antibodies. The levels of

antibodies to GBS types Ia, Ib, II and III varied widely in the different batches of IgG. High levels of antibodies to all types of GBS were not found in any of the batches. Seven of 53 batches contained high levels of antibodies against two different types, two batches against the combination Ia/Ib, two against Ib/III and the other three batches against the combinations Ia/III, Ib/II and II/III, respectively. The GBS antibody levels found in the different IgG batches and in 50 human blood donor sera, were compared (VII, fig. 2). The content of antibodies in 0.33 ml donor serum was compared to the results obtained with 0.85 mg of each of the commercial IgG preparations. Although the commercial IgG preparations contained 2.6 times more GBS-specific IgG than the donor sera under these conditions most donor sera fell within the range of the commercial preparations.

Unfortunately, none of the 53 commercial IgG preparations available seemed optimal for passive immunization against neonatal GBS disease. In a recent study, Schreiber and coworkers (1982) found human hyperimmune globulin preparations to contain ten times more protective antibodies against H. influenzae type b than conventional immunoglobulin. It was concluded that human hyperimmune globulin may be useful for the prevention of H. influenzae infections in high risk children. Our study suggests that similar specially designed hyperimmune globulin preparations against GBS would have to be manufactured for large scale prevention of neonatal GBS infections.

Bacteriological and immunological "follow up" in mothers after loss of fetus in GBS infection (VIII)

As already mentioned mothers of GBS-infected infants are generally deficient in type-specific serum antibodies against the type of GBS infecting their respective infant (Baker and Kasper, 1976a; Christensen et al, 1980a). However, not all infants of GBS carriers who are deficient in type-specific antibodies are infected (Baker and Kasper, 1976a; Christensen et al, 1980a), indicating that other hitherto unknown factors may predispose for the disease. Although we have no information on the outcome of future pregnancies in women who have previously given birth to a GBS-infected infant it is reasonable to assume that future fetuses are at risk provided that the mother still carries the same GBS type and exhibits an unaltered low serum antibody level against the organism. Paper VIII describes a program for preventing infection in infants of mothers fulfilling these criteria. For ethical reasons it was not possible to include a "control group" in this study.

In order to define a "risk" level of GBS antibodies in sera of parturient GBS carriers, specimens from 22 mothers, who had given birth to an infant with early onset GBS infection or who had experienced intrauterine fetal death due to GBS, were quantitated for type-specific IgG GBS antibodies using

radiolabelled protein A. All mothers except one, harboured the same type of GBS in the urogenital tract as isolated from their respective infant or fetus.

Nineteen of the 22 patients had low levels of antibodies against the infecting GBS type, when compared to urogenital carriers of GBS giving birth to healthy infants. This was in agreement with several other investigations, in which a variety of antibody-quantitation techniques were used (Baker et al, 1977; Christensen et al, 1980a; Hemming et al, 1976; Stewardson-Krieger et al, 1977; Vogel et al, 1979; II, III and VI).

Among the 19 patients with low serum levels of GBS antibodies three patients became pregnant again within one year of the loss of their infant or fetus. During the new pregnancy, all three patients were still carriers of the GBS type which caused the fatal infection. Their serum levels of typespecific antibodies remained low. Thus, all 3 belonged to the risk group previously defined and were included in a prevention program comprising the following steps. During the first trimester urethral and cervical specimens for culture of GBS and a serum sample for antibody quantitation were obtained. Treatment with fenoxymethylpenicillin (0.8 g twice daily) was started in the 28th week of pregnancy and continued until delivery. The effect of the penicillin on the urogenital flora was monitored weekly. At arrival to the labour ward, ampicillin was given intravenously. Immediately after birth specimens for culture were obtained from the infant and treatment of the infant with cefalexin was started and continued for 4 weeks.

The object of the prevention program was to suppress GBS in the maternal urogenital tract, thereby preventing the spread of GBS to the fetus in utero and minimizing the risk of contamination of the newborn during labour. Penicillin was chosen for prophylaxis because of its low toxicity and to avoid selecting resistant organisms, such as Klebsiella and Pseudomonas. During treatment with penicillin GBS was isolated on one occasion from each 2 patients, one of whom also had an E.coli.

All three patients went through a successful pregnancy without any complications. Four weeks after delivery they were again carriers of the GBS type isolated at the beginning of pregnancy. Up to date, another ten women fulfilling the risk group criteria have followed the prevention program, in each instance with successful result. These women also resumed their role as GBS-carriers after delivery.

In conclusion, it was possible to suppress GBS in the maternal urogenital tract with penicillin. However, although penicillin treatment was continued for 10-12 weeks, it was not possible to eradicate GBS in women who had been carriers for more than one

year and who lacked IgG antibodies to respective GBS type.

E. GENERAL DISCUSSION

Data concerning the severe GBS-infections included in the present thesis are summarized in Tables 2 and 3. In all, specimens from 43 infants and their mothers were studied. The cases were collected from various clinics in Sweden during 1977-1981.

Of the 43 cases, 36 were early onset and four late onset neonatal septicemia/meningitis whereas three infants died in utero. The severity of the infections is well illustrated by the mortality rate in the 43 live-born infants; 33 % (12 infants) died. The type distribution of the infecting GBS strains was: 8 type Ia (19.5%), 6 type Ib (15%), 8 type II (19.5 %) and 19 type III (46 %); two strains were not available for typing. In some materials from the United States as much as 90 % of the isolates from neonatal septicemia/meningitis has been reported to belong to type III (Wilkinson, 1978c).

Based on the material at hand, we have estimated that there are at least 110 cases of neonatal GBS septicemia/meningitis in Sweden annually or 1 per 1000 deliveries (Christensen et al, 1982a, b). Thus, it is evident that in Sweden GBS-infection is one of the most serious problems in the neonatal period. This is further underlined by the fact that a large proportion of the infants surviving neonatal septicemia/meningitidis suffer from neurological sequelae (Anthony and Okada, 1977). Add to this that intra-uterine infections leading to intra-uterine death also occur (VIII; Christensen et al, 1982a), and that approximately 0.7 % of all newborns contract GBS-pneumonia without septicemia (Christensen et al, submitted). Furthermore, for the individual mother having experienced the death of her child in an infection contracted due to her carriage of a bacterium, knowing that she still carries the organism and that the outcome of future pregnancies are uncertain, the successful prevention of future calamities are of paramount importance.

The antibody-response to bacterial carbohydrate and protein antigens in mothers of infants with severe GBS-infection

It was demonstrated in paper I, that carriage of GBS in the urogenital tract stimulates production of type-specific antibodies to the bacteria in adult women. However, papers II, III, VI and VIII showed that mothers of infants with GBS-septicemia/meningitis are exceptions to this rule. Further studies revealed that mothers of GBS-infected infants had low levels of serum IgG antibodies, not only to the type antigen of the infecting strain, but also to other GBS carbohydrate antigens as well as to carbohydrate antigens from other bacteria

(II). These findings led us to investigate the distribution of Gm-markers among mothers of GBS-infected infants (III) since there are several studies indicating an association between allotype immunoglobulin markers and antibody response to bacterial antigens. A marked deficit of G1m(1) individuals were found and the distribution of Gm-markers differed significantly from that in the general Swedish population. The results of papers II and III together indicate that mothers of infants with neonatal GBS-septicemia/meningitis are poor immunological responders to bacterial carbohydrate antigens on a genetic basis.

In the light of these findings it was natural to study whether mothers of GBS-infected infants were also deficient in IgG antibodies to protein antigens. This question is relevant to the discussion on the mechanism(s) responsible for the deficiency of maternal protective GBS antibodies. Among the protein antigens present in GBS, we decided to study the R-protein. Contrary to previous assumptions (Patterson and Hafeez, 1976) paper IV demonstrated that R-protein is of importance for the virulence of GBS type II. Thus, R-protein antiserum protected mice from infection with R-protein positive but not R-protein negative type II GBS. Our investigations (V) showed that R-protein is found in many GBS-strains: 51% of type II and 87% of type III strains carried the antigen. Tests of paired mother/infant strains gave concordant results as to the presence or absence of R-protein in the isolates. The epidemiological findings and the mouseprotection experiments indicated that R-protein might be a suitable candidate for the study of the humoral response to protein antigens in mothers of GBS-infected infants.

A method for quantitation of antibodies to R-protein in human sera was designed in paper VI. The method was based on absorption of the sera with an R-negative type III, strain followed by quantitation of antibodies to surface antigens of an R-positive type III strain, using radiolabelled protein A. Subsequently, mothers of infants infected with R-protein positive type II or type III strains were shown to have significantly lower levels of IgG antibodies to R-protein than mothers carrying R-positive strains but giving birth to healthy infants (VI). This finding suggests that R-protein is of importance for the virulence of GBS type II and type III. However, mothers of infants infected with R-negative strains did not show low levels of antibodies to R-protein i.e. these parturients produced "normal" amounts of antibodies to R-protein.

The difference in antibody response to bacterial carbohydrate and protein antigens support the hypothesis that the immunological aberration of mothers of infants with severe GBS-infections is mainly related to carbohydrate antigens. Such antigens have generally been assumed to be "thymus-

independent antigens" i.e. T-lymphocytes ("helper cells") are not involved in the antibody response against these antigens (Parrott and deSousa, 1971). In contrast, protein antigens are commonly characterized as "thymus-dependent".

It has been suggested that women could be vaccinated with purified type III carbohydrate antigen in order to prevent septicemia/meningitis with this type (Baker and Kapser, 1976a). Our studies indicate that women who may give birth to infants contracting GBS infection will not respond sufficiently to such an antigen. In support of this hypothesis, individuals with low preimmunization antibody levels against pneumococcal antigens generally respond poorly to pneumococcal vaccine (Baker et al, 1980). However, the possibility exists that a coupling of GBS carbohydrate antigens to a protein antigen might render the carbohydrate "thymus-dependent" and thereby more immunogenic in these women.

Prophylaxis against GBS neonatal septicemia/meningitis

It is still not possible to determine which measure will be most effective, economical and/or practical in the controlling of neonatal GBS-disease. However, there are several encouraging points which emerge from the results discussed above.

General penicillin-prophylaxis in all parturients or newborns for the prevention of neonatal GBS infection can not be recommended because of the risk of selecting penicillin-resistant organisms e.g. Enterobacteriaceae. Although in one study the incidence of neonatal GBS-septicemia was diminished by the administration of penicillin to all newborns, a simultaneous increase in infections caused by penicillin-resistant microorganisms was registered (Siegel et al, 1981). The possibility of preventing GBS by the use of local antiseptics in the vagina has so far not been studied.

It appeared from paper VII that commercially available gammaglobulin contain low levels of specific antibodies to GBS type antigens. However, specially designed gammaglobulin preparations could probably be manufactured by selecting donors with high levels of specific anti-GBS antibodies. Such a preparation could be administered to pregnant women e.g. from week 28 (to prevent intrauterine infection) and at delivery (to prevent neonatal infection). Another possibility would be to induce human cell lines to produce suitable antibodies.

Another form for prophylaxis is to define risk groups i.e. women at high risk of giving birth to infants contracting GBS infection. These parturients should then be handled according to well-defined prevention programs. Paper VIII represents the first attempt toward such an approach. Although parturients who have previously given birth to GBS-infected infants only

constitute a minority of the parturients at risk, it is especially important to avoid a repeated incident. Thus all efforts should be made to diagnose GBS neonatal and intra-uterine infections. In this context, sterile autopsies with extensive bacteriological investigation of perinatally dead infants are of utmost importance (Christensen, 1982).

It may be that by extending the studies in paper III to other Gm-markers or to other genetical markers would result in a more well-defined group of individuals at high risk of giving birth to infants contracting GBS-septicemia. However, there are also other possibilities for defining of risk groups.

One important approach is of course to detect parturients who are urogenital carriers of GBS i.e. a screening program for all women in pregnancy weeks 28 and 36. The early symptoms of neonatal GBS-septicemia are usually uncharacteristic and it is therefore of immediate clinical interest to know whether the mother is a GBS-carrier or not. In previous studies, pathological FHR-changes during labour and pH values below 7.20 during the first stage of labour were more often found among infants to urogenital GBS carriers than among infants to non-carriers (Christensen et al, 1980b). The registration of characteristic FHR-changes and low pH values in a GBS-carrying woman may be helpful for the rapid diagnosis of GBS infection in the infant.

Although many of the possibilities for preventive measures listed above are hypothetical, a few of them are available today. Thus, it is beyond doubt that screening of pregnant women for urogenital carriage of GBS is of great clinical significance and reasonable also from a cost-benefit point of view. Finally all efforts should be made to recognize neonatal GBS septicemia/meningitis and intrauterine infection in order to prevent the recurrence of the incident in future pregnancies.

Summary

During the first three decades of this century, group B streptococci (GBS) were generally considered harmless in man whereas the bacterium was known as an important agent in epidemic mastitis in cattle. In the following years sporadic cases of neonatal and puerperal infections were reported. The association between GBS and serious problems in the neonatal period was not recognized until the beginning of the 60ties.

Today, two major clinical forms of neonatal septicemia/meningitis are known: (1) the early onset form in which the infant falls ill within 10 days (usually 48 hours) of birth has its origin in contamination of the infant with GBS from the birth canal; and (2) the late onset form which is probably the result of a nosocomial spread of GBS in maternity wards. The annual rate of neonatal GBS infections in Sweden has been estimated to be at least 110 cases with a mortality of approximately 30%.

Until 1976, it remained unclear why only a few per cent of infants born to women who were urogenital carriers of GBS contracted septicemia/meningitis. Then a correlation between abnormally low levels of maternal IgG antibodies to GBS type III-antigen and infection in the newborn was described. This association has been confirmed by several investigators and the observation extended to three other GBS types, Ia, Ib and II.

Immunological response to bacterial carbohydrate antigens in mothers of infants with GBS-septicemia/meningitis

A correlation between antibodies to GBS type-antigens and urogenital carriage of the bacteria was demonstrated in Paper I. Parturients who were carriers of GBS generally had higher levels of type-specific antibodies than non-carriers, irrespective of the type of GBS isolated from the urogenital tract. Among the GBS carriers, the antibody levels were higher in younger parturients (20-29 years) than in older (30-39 years) whereas no such differences could be demonstrated among non-carriers.

Paper II confirmed previous studies demonstrating that mothers of GBS-infected infants had low levels of IgG-antibodies against the GBS type infecting the newborn child. However, these mothers, when compared to urogenital carriers of GBS giving birth to healthy infants, also showed low antibody levels against other GBS type-antigens. Furthermore, we quantitated IgG and IgM antibodies to S. typhi murium, S. enteritidis, Y. enterocolitica, F. tularensis as well as IgG antibodies to five types of S. pneumoniae capsular polysaccharide. A greater number of mothers of GBS-infected infants, as compared to GBS-carriers giving birth to healthy infants, had IgG antibodies below a selected limit for 8 of 9 carbohydrates. On the other hand, in the first group high IgM levels against Salmonella enteritidis antigen were common.

Several investigations have indicated an association between Gm allotypes and immune response to bacterial antigens. The distribution of Gm-allotypes among mothers of GBS-infected infants was studied and compared to the distribution in the Swedish population (paper III). The number of individuals with the phenotypes Gm(1,-5), Gm(1,5) and Gm(-1,5) among the 34 mothers studied were 5, 7 and 22, respectively, whereas the expected distribution was 4.15, 15.46 and 14.40, respectively ($p < 0.01$). This indicated that mothers of GBS-infected infants were poor IgG responders to bacterial carbohydrate antigens on a genetic basis. It was concluded that such patients would probably not respond satisfactorily to a vaccine against GBS based on purified carbohydrate antigens.

Epidemiological and immunological aspects of R-protein in GBS

For the study of the IgG response to bacterial protein antigens we selected R-protein of GBS.

A monospecific R-protein antiserum gave clear-cut protection of mice against type II isolates containing R-protein but not against GBS strains of the same type lacking R-protein (IV). Phagocytosis experiments with mouse macrophages confirmed that type II, R-positive strains were opsonized by R-protein antiserum. Thus, in contrast to the opinion of previous investigators, the R-protein in R-protein carrying GBS type II, was found to be important for the virulence.

A material of 241 clinical isolates of GBS was surveyed for the presence of R-protein (V). It was detected in 51% of type II and 87% of type III strains but not in types Ia and Ib. Tests of paired strains from mother/infant showed concordant results with respect to the presence or absence of R-protein. The protein was detected in one of two type II and in 12/14 type III strains isolated from cases of neonatal septicemia/meningitis.

In paper VI a method for determination of IgG antibodies to R-

-protein was developed. The antibody levels to R-protein among mothers of infants infected with R-protein positive type II strains, were significantly lower than in carriers of R-protein positive type II GBS giving birth to healthy infants. A similar result was obtained with respect to R-protein positive type III GBS. However, no difference was found with respect to R-protein antibodies between the study group and the controls, the latter having been exposed to R-protein negative type II and type III strains. It was concluded that in R-protein positive strains the protein played a role for the virulence of the bacterium. However, mothers of GBS-infected infants did not show uniformly low R-protein antibody levels in contrast to the general picture obtained with carbohydrate antigens. These findings indicated that the relative inefficiency of the IgG-production against GBS-antigens could be ascribed to "thymus-independent" antigens.

Prevention of neonatal GBS-disease

Our results did not favour vaccination with purified GBS carbohydrate antigens as a measure for prevention of neonatal GBS septicemia/meningitis. Another approach might be to protect the infant at risk by passive immunization of the mother before delivery. In order to study this possibility, 53 commercial batches of IgG were quantitated for type-specific GBS-antibodies (VII). The levels of antibodies to types Ia, Ib, II and III varied widely in the different batches. High levels of antibodies to all types were not found in any of the batches. The results obtained with the commercial preparations were compared to the antibody levels in 50 human blood donor sera. None of the commercial preparations contained more type-specific IgG than donor sera. The findings indicated that special hyperimmune IgG preparations based on selected donors would have to be designed for prophylactic use.

Among parturient GBS-carriers one can distinguish a risk group of pregnant women on the following criteria: (1) women who have previously given birth to an infant contracting GBS-septicemia/meningitis and (2) who are still urogenital carriers of GBS and (3) who show unaltered low serum antibody level against GBS. Paper VIII describes a prevention program including (1) treatment with penicillin orally from pregnancy week 28; (2) weekly monitoring of the urogenital flora; (3) administration of high doses of penicillin intravenously during delivery; (4) treatment of the infant with antibiotics for 4 weeks after birth. Three women are described who successfully followed this programme. Up to now, 13 healthy infants have been born to mothers who had previously given birth to infants contracting severe GBS-infection.

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Appendix (I-VIII)

Type-specific Serum Antibodies against Group B Streptococci among Pregnant Women: Relation to Urogenital Carriage and Age

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ABSTRACT. Serum levels of type-specific antibodies against group B streptococci (GBS) were investigated in 53 pregnant urogenital carriers of GBS and in 20 pregnant non-carriers. The distribution of serotypes of GBS isolated from the urogenital tract of the 53 culture-positive patients was: type Ia, 20 patients (38%), type II, 10 (19%), and type III, 23 (43%). Antibodies to GBS types Ia, Ib, II and III were quantitated by the use of radiolabelled protein A. It was found that the pregnant GBS carriers had in general higher levels of type-specific antibodies to GBS than non-carriers, irrespective of the type of GBS harboured in the urogenital tract. Furthermore, it was found that younger GBS carriers (20 to 29 yr of age) had higher serum levels of type-specific antibodies than older carriers (30 to 39 yr), a difference that did not occur among the non-carriers.

INTRODUCTION

Group B streptococci (GBS) are a well-known cause of serious infections in newborns (1, 11, 13). It has been shown that mothers of infants who contract neonatal GBS sepsis/meningitis are deficient in antibodies against the type of GBS causing the infection (2, 5, 14, 19). In this context, it is of obvious interest to investigate the relation between urogenital carriage of GBS and the production of type-specific serum antibodies.

In a previous study (6) upper respiratory tract spread of GBS type Ib in a kindergarten was found to result in the development of type-specific serum antibodies against type Ib among the staff members. Production of GBS antibodies can also be stimulated through urogenital carriage of GBS (7); female patients attending a venereological out-clinic were found to have higher levels of type-specific GBS antibodies than patients at a gynaecological out-clinic and girls in the age range 10-12 yr.

The present study concerned type-specific serum antibodies against GBS in relation to urogenital carriage of GBS among pregnant women. Furthermore, the relation of antibody levels to age and parity was investigated.

MATERIALS AND METHODS

Patient sera. Sera from 53 patients, who were urogenital carriers of GBS in pregnancy week 36 and at delivery (study group), and sera from 20 parturients who were culture-negative for GBS in week 36 and at delivery (control group) were investigated for antibodies against GBS types Ia, Ib, II and III. These parturients were selected from among 143 GBS carriers and 157 culture-negative patients who were described elsewhere (10). The study group fulfilled the following criteria: they carried the same type of GBS in week 36 and at delivery, and they gave birth to healthy infants after normal pregnancies. The mean age of the women was 28.5 yr (range 20-39). The control group consisted of 10 patients aged 20-29 yr and 10 patients aged 30-39; they were selected from 157 culture-negative parturients matched in parity with the study group. The patients in the control group had also borne healthy infants.

Bacteriological examinations. Culturing, grouping and typing of GBS were performed as previously described (4).

The distribution of serotypes of GBS present in the urogenital tract of the 53 culture-positive patients was: type Ia, 20 patients (38%), type II, 10 (19%) and type III, 23 (43%). Carriers of type Ib were not included.

Quantitation of serum antibodies to GBS types Ia, Ib, II and III. Antibodies to GBS types Ia, Ib, II and III were measured with radiolabelled protein A as described (5). In brief, the serum to be tested was absorbed with type II GBS before determination of antibodies against types Ia,

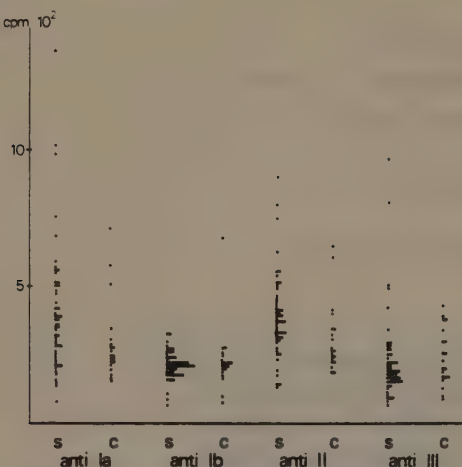


Fig. 1. Test of sera from pregnant urogenital GBS carriers (S) and pregnant non-carriers (C). Ordinate: uptake of radiolabelled protein A on the bacteria after addition of sera. Anti Ia, Ib, II and III indicate those bacteria against which antibodies were measured.

Ib and III, and with type III GBS when antibodies to type II were being measured. The absorbed serum was mixed with a standard suspension of the type of GBS to which antibodies were to be measured. The quantity of antibodies bound to the surface of the bacteria after washings was determined with protein A, which reacts with the Fc part of human IgG of subclasses 1, 2 and 4 (16). The antibody level was expressed as the radioactive protein A in counts per min (cpm) bound to the bacteria after coating with serum.

Statistical analysis. The Student's *t*-test for non-pair data was used throughout the study.

RESULTS

Relation between GBS carriage and serum levels of type-specific antibodies against GBS

Serum levels of antibodies against type Ia and type II were significantly higher in the study group than in the control group ($p < 0.05$) (Fig. 1). By contrast there was no inter-group difference in serum levels of antibodies against types Ib and III.

The levels of antibodies to type Ia were found to be significantly higher among carriers of type Ia and of type III than among the carriers of type II and the controls ($p < 0.05$). Otherwise, no correlation could be found between serum levels of antibodies and

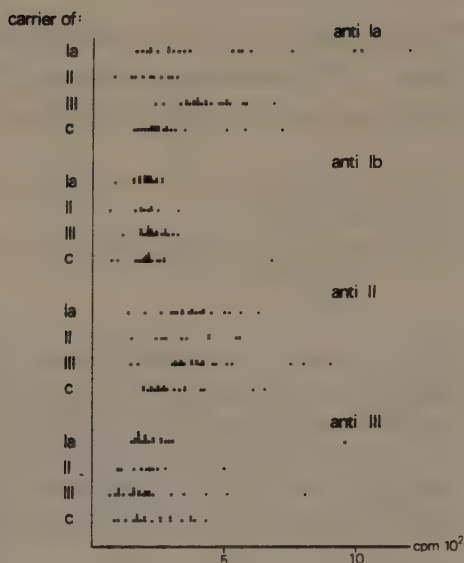


Fig. 2. Test of sera from pregnant carriers of GBS types Ia, II and III, and pregnant non-carriers (C). The sera were tested for antibodies against Ia, Ib, II and III. Abscissa: uptake of radiolabelled protein A on bacteria after addition of sera.

the type of GBS present in the urogenital tract (Fig. 2).

Relation between age and serum levels of type-specific antibodies against GBS

34 of the parturients in the study group were between 20 and 29 yr of age, while 19 were between 30 and 39. In the control group, 10 patients were between 20 and 29 yr and 10 between 30 and 39. There was no difference in the distribution of serotypes isolated from the GBS carriers in the two age groups; among the younger patients, 15 harboured type Ia, 5 type II and 16 type III. GBS carriers aged 20–29 yr had higher levels of antibodies against types Ib and II than GBS carriers aged 30–39 yr ($p < 0.05$) (Fig. 3). The study group members aged 20–29 yr also showed higher serum levels of antibodies against types Ia and II than did the controls in this age range ($p < 0.05$). Among the older GBS carriers, a higher level of antibodies to type Ia was found than among the older controls ($p < 0.05$). There was no difference in antibody level to any

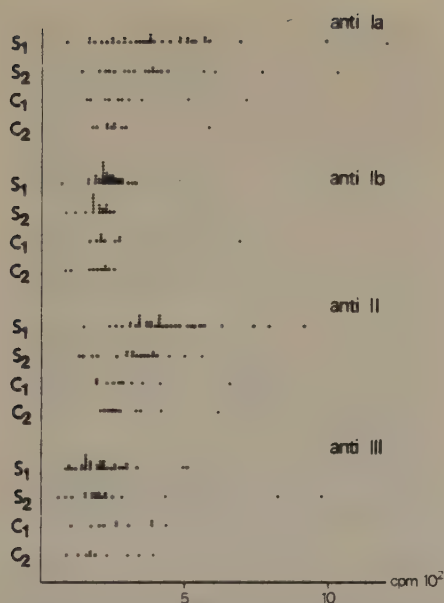


Fig. 3. Test of sera from pregnant GBS carriers aged 20–29 yr (S_1) and 30–39 yr (S_2); and from pregnant non-carriers aged 20–29 yr (C_1) and 30–39 yr (C_2). The sera were tested for antibodies against Ia, Ib, II and III. Abscissa: uptake of radiolabelled protein A on bacteria after addition of sera.

type of GBS among the younger and older subjects in the control group.

The carriers of type Ia in the age group 20–29 yr had higher levels of antibodies to type Ia and type II than the carriers of type II in the same age range ($p < 0.05$) (Fig. 4). Moreover, the younger type III carriers had higher levels of antibodies to type Ia than had carriers of type II and the controls, and also higher levels of type II antibodies than the controls ($p < 0.05$).

25 GBS carriers and 10 non-carriers were primigravidae. There was no correlation between number of pregnancies and the patients' age. No differences in serum levels of type-specific antibodies against GBS were found between the primigravidae and multigravidae either in the study group or in the control group.

DISCUSSION

It was found in this investigation that GBS carriers had higher serum levels of antibodies to GBS types

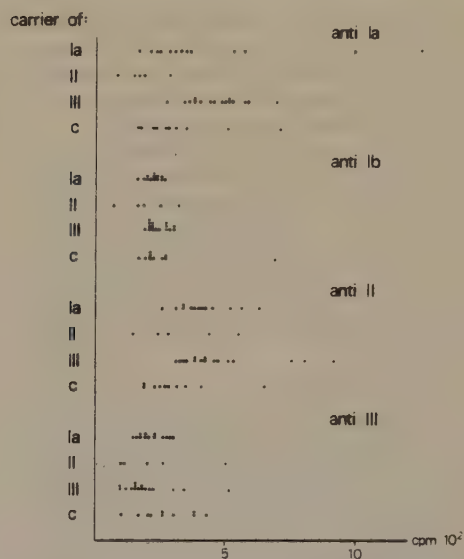


Fig. 4. Test of sera from pregnant carriers of GBS types Ia, II and III, and pregnant non-carriers (C) in the age group 20–29 yr. The sera were tested for antibodies against Ia, Ib, II and III. Abscissa: uptake of radiolabelled protein A on bacteria after addition of sera.

Ia and type II than had non-carriers. The levels of antibodies were generally higher among the younger GBS carriers (20–29 yr) than among the older ones (30–39 yr), whereas no such differences were shown among the two age groups in the controls. The serum levels of type-specific antibodies could not be related to the type of GBS isolated from the urogenital tract, except for type Ia carriers who had higher levels of type Ia antibodies than the controls. Vogel et al. (19) reported that carriers of type Ia had higher levels of type Ia antibodies than had non-carriers, when using an immunofluorescence method for the detection of antibodies. Using the chick embryo protection test, they found that carriers of type II and of type III had more protective antibodies against types II and III than non-carriers. Beachler et al. (3) reported that the radioactive antigen-binding assay for type III antibody determination, detected more antibodies in the carriers of this type than in non-carriers.

In an earlier investigation (8) we found that levels of type-specific antibodies to GBS were not related to site of colonization. Thus, the parturients har-

pouring GBS in the urethra only and combined urethral and cervical carriers did not differ as regards antibody levels. Likewise, it was found that the levels of type-specific antibodies did not influence the colonization of the newborns (8).

Several studies (9, 12, 15, 17) have shown that GBS could be isolated more frequently from the urogenital tract of females attending a venereological department than from other groups of women. We have found (7) that such patients have higher levels of type-specific serum antibodies to GBS than other groups of individuals, indicating that urogenital carriage of GBS encourages antibody production.

In the present study it was found that the younger GBS carriers had higher serum levels of antibodies to GBS than the older ones, although antibody levels were not correlated to the number of pregnancies experienced by the patients. This may indicate a more extensive spread of GBS among humans in this age group, or that antibodies to GBS in serum diminish with age. Higher concentrations of antibodies against *Chlamydia trachomatis* have also been found in younger age groups (18; Mårdh, personal communication).

From the present and previous studies (6, 7) it can be concluded that stimulation of the production of type-specific serum antibodies against GBS in man can occur via upper respiratory tract colonization and through urogenital colonization. The present study showed that pregnant GBS carriers generally had higher levels of type-specific antibodies to GBS than non-carriers, irrespective of the type of GBS harboured in the urogenital tract. This indicates that the GBS carriers had been exposed to various types of GBS, and not only to that one detected.

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Mothers of Infants with Neonatal Group B Streptococcal Septicemia Are Poor Responders to Bacterial Carbohydrate Antigens

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Abstract. Serum antibodies against various carbohydrate antigens were studied in 16 mothers of infants with serious infections caused by group B streptococci (GBS) (the study group), and compared with a control group of 29 urogenital carriers of GBS who gave birth to neonatally healthy infants. Using a radioimmunoassay for the determination of antibodies to GBS types Ia, Ib, II and III, it was found that the study group had significantly lower levels of IgG antibodies to each of the 4 GBS types than the control group. The IgG levels against *Salmonella* BO and DO, *Yersinia enterocolitica* 03, *Francisella tularensis* and *Streptococcus pneumoniae* types 3, 6, 9, 19 and 23 purified carbohydrate antigens were determined using an ELISA technique. Significantly more individuals in the study group than in the control group had low levels of IgG antibodies against 8 of 9 carbohydrate antigens. No difference was found in IgM levels against 3 of 4 antigens studied, while the study group showed significantly more IgM antibodies against *Salmonella* DO than the controls. These results indicate that mothers of GBS-infected infants might be poor IgG antibody responders to bacterial carbohydrate antigens in general.

Introduction

The importance of group B streptococci (GBS) as a cause of serious infections in newborn infants is well known [1]. In 1976, Baker and Kasper [4] reported the correlation between low maternal levels of antibodies to the type III polysaccharide antigen, and the risk of serious disease caused by organisms of this serotype in newborns. This was later confirmed and extended to other serotypes by a number of investigators, using a variety of antibody quantitation methods [6, 9, 17].

As a consequence, vaccination with GBS-type antigens has been suggested [3]. In this context, it is of obvious interest that Siber et al. [16] reported a correlation between preimmunization serum IgG2 concentrations and the antibody response to bacterial polysaccharide vaccines (from pneumococci and *Haemophilus influenzae*). The findings indicated that patients with low levels of IgG2 are poor responders to the polysaccharide vaccines. Supporting this view, Baker et al. [5] found that the preimmunization levels of antibody to GBS type III influenced the immune response to the antigen.

These findings raise the question whether mothers of GBS-infected infants, on a genetic basis, are poor responders to polysaccharide antigens. In the present work, we tested sera from such mothers against a panel of bacterial carbohydrate antigens. Our results indicate that a number of these women have low levels of IgG antibody against the antigens in general, while the IgM levels were found to be at least as high as in the controls.

Materials and Methods

Patients

Study Group. Sera from 15 mothers of infants who contracted GBS neonatal sepsis and/or meningitis, and from 1 patient who experienced intrauterine fetal death due to GBS, were investigated. The sera were obtained either during the first trimester of pregnancy (taken for routine testing of rubella antibodies; 5 sera) or within 1 month after delivery (11 sera).

GBS were isolated at sterile autopsy [7] from all inner organs of the antenatally dead fetus, and from blood and/or cerebrospinal fluid of the infected infants. 2 of these strains belonged to type Ia, 4 to type Ib, 2 to type II and 8 to type III.

12 infants contracted their infection within 48 h of birth, while 3

Table 1. Serum levels of IgG and IgM antibodies against bacterial carbohydrate antigens other than GBS antigens in mothers of GBS-infected infants compared with controls

Bacterial antigen used	Antibody concentrations numerical geometric mean (95% confidence interval)	
	study group (n = 16) ^a	control group (n = 29) ^a
IgG antibody		
<i>Salmonella</i> BO	230 (39–421)	364 (17–711)
<i>Salmonella</i> DO	269 (27–511)	373 (33–713)
<i>Y. enterocolitica</i> 03	375 (57–693)	504 (147–861)
<i>F. tularensis</i>	493 (113–872)	516 (237–795)
<i>S. pneumoniae</i> type 3	428 (67–789)	621 (0–1,319)
<i>S. pneumoniae</i> type 6	1,320 (305–2,335)	1,679 (778–2,580)
<i>S. pneumoniae</i> type 9	1,086 (501–1,671)	1,482 (680–2,284)
<i>S. pneumoniae</i> type 19	1,334 (516–2,152)	1,832 (904–2,770)
<i>S. pneumoniae</i> type 23	841 (306–1,375)	1,112 (550–1,674)
IgM antibody		
<i>Salmonella</i> BO	228 (62–394)	160 (49–271)
<i>Salmonella</i> DO	134 (65–203)	114 (54–174)
<i>Y. enterocolitica</i> 03	141 (47–235)	119 (59–179)
<i>F. tularensis</i>	167 (74–260)	125 (56–194)

^a According to Student's *t* test, no significant difference was found between the study group and the control group.

infants fell ill after 1 week of life. The overall mortality rate was 4 out of 15 (26.7%). All mothers were urogenital carriers of the strain infecting their infants.

Control Group. The control group consisted of 29 parturients who were urogenital carriers of GBS; their infants showed no signs of infection during the neonatal period. Sera were obtained from the women during pregnancy (routine test for rubella antibodies). 10 controls were carriers of type Ia GBS, 5 of type Ib, 5 of type II and 9 of type III.

Bacteriological Examinations

GBS isolated from blood and/or cerebrospinal fluid of the infants and from the inner organs of the fetus were identified, grouped and typed as described in an earlier work [6]. Urethral and cervical specimens were taken as previously described using a selective method [2].

Quantitation of Serum Antibodies

Type-Specific GBS Antibodies. Antibodies to GBS types Ia, Ib, II and III were measured with radiolabelled protein A as earlier described [6]. In brief, the serum to be tested was absorbed with type II GBS before determination of antibodies against types Ia, Ib and III, and with type III GBS when antibodies to type II were being measured. The absorbed serum was mixed with a standard suspension of the type of GBS to which antibodies were to be measured. The quantity of antibodies bound to the surface of the bacteria after

washings was determined with protein A which reacts preferentially with the Fc part of human IgG of subclasses 1, 2 and 4 [11]. The antibody level was expressed as the radioactive protein A in counts per minute (cpm) bound to the bacteria after coating with serum.

Antibodies to Other Carbohydrate Antigens. Antibodies to *Salmonella typhimurium* [O-antigens 4, 12 (BO)], *Salmonella enteritidis* [0:9, 12 (DO)], *Yersinia enterocolitica* type 03, and *Francisella tularensis* lipopolysaccharides (LPS) as well as *Streptococcus pneumoniae* types 3, 6, 9, 19 and 23 capsular polysaccharides were estimated using a microplate ELISA [16]. The LPS antigens were prepared as earlier described [16]; the pneumococcal antigens were obtained from Merck, Sharp & Dome, Rahway, N.J., USA. Coating of the microplates with LPS antigens was done in a buffer of pH 9.6, whereas for the pneumococcal antigens, optimal coating was done in a buffer of pH 8.5. Monospecific swine antihuman IgG- and IgM-alkaline phosphatase conjugates were purchased from Orion Diagnostica, Helsinki, Finland. IgG antibody titers were most efficiently estimated at 25°C, whereas IgM titers were estimated at 37°C. Results were determined photometrically at 405 nm using a Titertec Multiscan (Flow Laboratories, Ingelwood, Calif., USA). Titers are expressed as the absorbance value of the 1:1,000 serum dilution multiplied by the dilution factor ($\times 1,000$).

Statistical Analysis

The χ^2 -test (the exact method) and Student's *t* test for nonpaired data were used.

Individuals with low antibody concentrations

selected limit of antibody concentration	within the selected limits, n		p value (χ^2 -test)
	study group (n = 16)	controls (n = 29)	
< 80	3	0	0.037
< 80	3	0	0.037
< 120	5	0	0.004
< 120	4	0	0.012
-	-	-	-
< 610	5	1	0.015
< 600	6	2	0.015
< 800	6	2	0.015
< 500	6	2	0.015
-	-	-	-
* > 190	5	1	0.015
-	-	-	-
-	-	-	-

Results

Serum Antibody Levels against GBS-Type Antigens

Antibodies against types Ia, Ib, II and III GBS were measured in the sera of the 16 mothers with infected infants, and compared with the levels in the sera from 29 urogenital carriers of GBS who gave birth to healthy infants. It was found that the study group had significantly lower levels of antibodies than the control group against each of the types (Student's t test; for type Ia: $p < 0.05$, for types Ib, II and III: $p < 0.001$; fig. 1). Within the study group, the 3 highest levels of antibodies against each type were found in 5 different patients (fig. 1).

The levels of anti-Ia antibodies in sera from mothers of Ia-infected infants were also compared to those found in the controls carrying Ia GBS at the delivery, etc. (fig. 2). 14 of 16 mothers of infected infants had low levels of antibodies against the infecting type compared with the controls (fig. 2). Of the remaining 2 patients, 1 suffered from juvenile diabetes while the



Fig. 1. Test of sera from mothers of GBS-infected infants (P) and from urogenital carriers of GBS giving birth to healthy infants (C). Each serum was tested against GBS types Ia, Ib, II and III. Ordinate: uptake of radiolabelled protein A on the bacteria after addition of sera. Ia, Ib, II and III indicate the bacteria against which antibodies were measured.

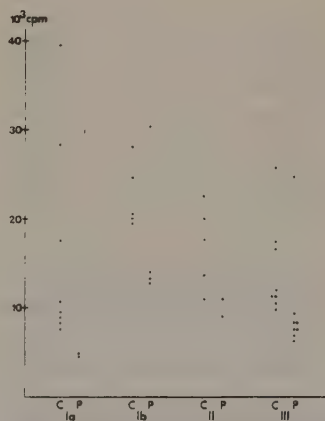


Fig. 2. Test of sera from mothers of GBS-infected infants (P) and from urogenital carriers of GBS giving birth to healthy infants (C). Each maternal serum was tested against the type of GBS infecting the corresponding infant, or that found in the urogenital tract of each mother giving birth to a noninfected infant. Ordinate: uptake of radiolabelled protein A on the bacteria after addition of the sera. Ia, Ib, II and III indicate the bacteria against which antibodies were measured.

* corrected compared to the original

her was heavily ABO-immunized, necessitating several blood-exchange transfusions in her infant immediately after birth. It was also noteworthy that these 2 patients had comparably high levels of antibodies against all four types of GBS.

Serum Antibody Levels against Bacterial Carbohydrate Antigens Other Than GBS-Type Antigens

The study group sera and control sera were tested for IgG antibodies against several purified carbohydrate antigens, including *Salmonella* BO and DO, *Y. enterocolitica* 03, *F. tularensis* and the pneumococcal capsular antigens of types 3, 6, 9, 19 and 23 (table I). Furthermore, IgM antibodies were measured against *Salmonella* BO and DO, and the *Yersinia* and *Francisella* antigens (table I). The numerical geometric mean IgG concentrations was lower in the study group than in the control group, for all 9 antigens; in contrast, the numerical mean of IgM concentrations against all 4 antigens tested was higher in the study group. When comparing the Gauss distributions of IgG and IgM levels for each antigen, no significant difference was found between the two groups of sera ($p > 0.05$; Student's *t* test). However, the IgG levels against all pneumococcal antigens taken together were lower in the study group than in the controls ($p < 0.05$; Student's *t* test). Similarly, comparison of the IgG antibodies against all gram-negative antigens (*Salmonella*, *Yersinia* and *Francisella*) showed significantly lower values for the study group ($p < 0.05$; Student's *t* test). The IgM levels against all the gram-negative antigens taken together did not differ significantly, using Student's *t* test.

In order to test the hypothesis that the study group might include individuals with very low IgG or very high IgM levels, the χ^2 -test (exact method) was applied to those of the study group and control group, respectively, showing levels above and below selected antibody concentrations against each of the carbohydrate antigens. Using this method, we found significantly more individuals in the study group than in the control group with low IgG levels against 8 of 9 carbohydrate antigens (table I). In all, 9 of the patients in the study group, and 2 of the controls were found to have IgG antibody levels below the selected limits. Each of these individuals had low levels against at least 3 antigens. Finally, the study group had significantly more individuals with high IgM levels against *Salmonella* DO antigen.

The 2 patients from the study group with high levels of specific antibodies against the infecting type of GBS had IgG antibody concentrations above the selected limits for all carbohydrate antigens listed in table I.

Discussion

In this study, the capacity to produce antibodies against bacterial carbohydrate antigens was investigated in mothers of GBS-infected infants. Earlier studies have shown that these mothers are deficient in type-specific serum antibodies against the strains infecting their infants [4, 6, 9, 17]. This finding was confirmed, using radiolabelled protein A which detects antibodies primarily of IgG subclasses 1, 2 and 4. 14 of 16 mothers of GBS-infected infants had low levels of IgG antibody against the type of GBS infecting their respective infant. Furthermore, it was found that the mothers of GBS-infected infants had lower levels of IgG antibodies against all types of GBS in comparison with 29 urogenital carriers of the bacteria who gave birth to healthy infants (fig. 1). This correlation has not previously been reported. 2 mothers had high levels of antibodies against the infecting type, and also against other types of GBS. This indicates that obstetrical and neonatal complications may accidentally overcome the immune barrier. Such complications, and the degree of contamination of the infant, do probably contribute to the development of neonatal GBS disease, since not all carriers with low antibody levels give birth to infants who become infected in the neonatal period. We conclude that mothers of GBS-infected infants are relatively deficient in type-specific antibodies not only to the infectious strain but also to other GBS carbohydrate antigens. We have recently described 3 such mothers who had persistently low IgG levels although they carried GBS for at least 1 year [8].

These findings forced us to investigate serum levels of antibodies against 9 other bacterial carbohydrate antigens. The study group (16 mothers of GBS-infected infants) and the controls (29 urogenital carriers of GBS who gave birth to healthy infants) were compared. The IgG levels against a group of 5 pneumococcal antigens and against 4 gram-negative antigens were lower in the study group than in the controls. For each of the 9 antigens, a lower limit of IgG antibody response was selected (table I). More individu-

als from the study group than from the control group had IgG antibodies below the selected limit for 8 of 9 carbohydrate antigens. In all, 9 of 16 in the study group and 2 of 29 controls had low levels of IgG antibodies against at least 3 carbohydrate antigens. It is highly probable that the individuals investigated had been exposed to the test antigens, not so much through infections with the corresponding organism, as via asymptomatic colonization of bacteria of the normal flora possessing cross-reactive antigens. Thus, our findings indicate that a majority, 9 of 16, of mothers of GBS-infected infants are low responders to bacterial carbohydrate antigens.

Interviews with the mothers of GBS-infected infants did not reveal that they had experienced an excess of infections with bacteria carrying carbohydrate capsules. This might have several explanations; first of all, IgG antibodies against these antigens were not completely absent. Furthermore, our results might indicate that they produced more IgM antibodies against bacterial carbohydrate antigens than mothers giving birth to healthy infants. Thus, more individuals with high IgM levels against *Salmonella* DO antigen were found in the study group than among the controls. It is conceivable that mothers of GBS-infected infants compensate their relative IgG anticarbohydrate deficiency through production of IgM antibody; since IgG (but not IgM) passes the placenta, their infants (but not they themselves) would be susceptible to GBS infections. This theory would also be interesting to investigate with neonatal *E. coli* infections, in which strains of capsular carbohydrate K1 dominate [14].

Several investigations have indicated that IgG antibody directed against carbohydrate antigens consists primarily of IgG2 [13, 16, 18]. Pandey et al. [12] reported an association between immunoglobulin allotypes and immune response to *H. influenzae* and meningococcus carbohydrates. These findings might indicate that the antibody response to carbohydrate antigens is regulated by genetic factors. Taken together with the findings in the present investigation, it opens up a prospect of detecting women who run a high risk of having GBS-infected infants, by the use of genetic markers. On the other hand, our results indicate that vaccines composed of purified GBS carbohydrate antigens would be less useful for the prevention of perinatal GBS disease, though coupling of the carbohydrate antigens to proteins might overcome the difficulties [15]. Nevertheless, definition of a high-risk

group by means of genetic markers would seem to be realistic, in view of the present findings; serious GBS infections might be prevented by specific γ -globulin or antibiotics in this group.

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SHORT COMMUNICATION

ASSOCIATION BETWEEN MATERNAL *Gm* ALLOTYPE AND NEONATAL SEPTICAEMIA WITH GROUP B STREPTOCOCCI

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SUMMARY

Thirty-four mothers to infants seriously infected with group B streptococci (GBS) were investigated for G1m(1) and G3m(5) allotype markers. The frequency of Gm(1, -5) was 14.7%, of Gm(1, 5) 20.6% and Gm(-1, 5) 64.7%. There was a marked deficit of Gm(1) individuals and the distribution significantly differed from that in the normal Swedish population.

INTRODUCTION

The importance of group B streptococci (GBS) as a cause of serious infections in newborn infants is well established (Baker, 1977). In 1976, Baker & Kasper reported a correlation between low maternal levels of antibodies to the type III GBS polysaccharide antigen and the risk of serious disease caused by organisms of this serotype in newborns. This was later confirmed and extended to other serotypes, using a variety of antibody quantitation methods (Christensen *et al.*, 1980, 1981; Hemming *et al.*, 1976; Vogel *et al.*, 1980). These results indicate that mothers of GBS-infected infants might be poor IgG-antibody responders to these bacterial carbohydrate antigens.

Several investigations indicate an association between *Gm* allotypes and particular immune responses (Mackay *et al.*, 1975; Nevo, 1974; Pandey *et al.*, 1979; Rivat *et al.*, 1978; Wells *et al.*, 1971) as well as between *Gm* allotypes and ability to survive infectious diseases (Schanfield, 1975; de Vries *et al.*, 1979). The present paper concerns the distribution of *Gm* allotypes in Swedish mothers to GBS-infected infants. Furthermore, we have studied the possible association between blood group B in the mother and GBS-infection in the infant, since maternal blood group B has been reported to be a risk factor in this disease (Regan *et al.*, 1978; Reid & Lloyd, 1980).

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MATERIALS AND METHODS

Patients

All sera available to us, from mothers of infants who contracted GBS neonatal sepsis and/or meningitis, (thirty-two patients) and from patients who experienced intrauterine foetal death due to GBS (two patients), were investigated. The sera were obtained either during the first trimester of pregnancy (four sera) or within one month after delivery (thirty sera). All mothers had been born in Sweden.

GBS were isolated at sterile autopsy (Christensen *et al.*, 1981) or from blood and/or cerebrospinal fluid of the infected infants. Seven of these strains belonged to Type Ia, four to Type Ib, eight to Type II and thirteen to Type III, while two strains were not available for typing. Twenty-nine infants contracted their infection within 48 hr of birth, while three infants fell ill after one week of life. The overall mortality was 26%. All mothers were urogenital carriers of the strain infecting their respective infants.

Data concerning eighteen of these patients have been published elsewhere (Christensen *et al.*, 1981).

Quantitation of antibodies to GBS Types Ia, Ib, II and III; Gm-typing; blood grouping

These tests were performed as described (Christensen *et al.*, 1980). The antibody levels measured in the patients' sera were compared with those in sera from mothers found to be harbouring GBS Types Ia, Ib II and III in the urogenital tract and who gave birth to infants without signs of infection. The antibody levels among the controls were measured against the respective type harboured in the urogenital tract.

Gm-typing for G1m(1) and G3(5) was performed according to standard methods (see Grubb 1970). The gene frequencies for these markers in the Swedish population are based on an analysis of 2.917 individuals (Statens Rättskemiska Laboratorium, Sweden; courtesy of T. Swan, Linköping). These frequencies are $Gm^1 = 0.3494$ and $Gm^5 = 0.6507$.

ABO blood group typing was performed by standard methods. The frequencies of blood group antigens in Sweden (Table 3) are based on studies of 137,762 individuals (Mourant *et al.*, 1976).

RESULTS

Table 1 shows the serum antibody levels in thirty-two mothers against the respective types of GBS infecting their infants. Twenty-eight of these mothers showed very low levels of GBS-antibodies compared to controls who were carriers of GBS but gave birth to non-infected infants. On the other hand, four gave markedly high antibody levels.

The frequencies of types Gm(1, -5), Gm(1, 5) and Gm(-1, 5) among the thirty-four mothers of GBS-infected infants were compared to the frequencies expected in the Swedish population (Table 2). The distribution of Gm types in these mothers differed significantly from the distribution in the Swedish population at large. In these mothers, there was a surplus of individuals possessing Gm^5 and, correspondingly, a deficit of those with Gm^1 . Notably, the homozygotes (i.e. Gm(1, -5) and Gm(-1, 5)) exceeded the number expected. Among the four sera with high type-specific levels against GBS, two were Gm(-1, 5) and the other two Gm(1, -5) phenotypes.

The ABO blood group distribution among the mothers of GBS-infected infants did not differ from the distribution expected in the Swedish population (Table 3).

Gm(1) deficit and neonatal septicaemia

TABLE 1. Test of sera from mothers of infants with GBS sepsis and/or meningitis, compared to sera of GBS-carriers giving birth to healthy infants*

Antibodies determined against Type	Antibody levels in control sera, range (mean), in c.p.m.	Antibody levels (CPM) in mothers of GBS-infected infants
Ia	8.100-17.000 (11.100)	6 < 9.000 1 > 20.000
Ib	17.000-28.000 (20.760)	2 < 17.000 2 > 28.000
II	12.500-23.000 (18.750)	8 < 11.000
III	9.000-31.000 (17.300)	12 < 9.000 1 > 29.000

* Results are shown for test against the type infecting the respective infant or harboured in the urogenital tract of the control. Sera from two patients were not tested since the types of GBS infecting their infants were unknown.

TABLE 2. Gm phenotypes in mothers to GBS-infected infants

Phenotype	Observed		Expected		χ^2
	No.	Proportion	No.	Proportion	
Gm (1, -5)	5	0.1471	4.15	0.1220	0.17
Gm (1, 5)	7	0.2059	15.46	0.4547	4.63
Gm (-1, 5)	22	0.6471	14.40	0.4234	4.01
					$\Sigma \chi^2 = 8.81$
					$P < 0.01$ for d.f. = 2

TABLE 3. Blood groups in mothers to GBS-infected infants

Blood group	Observed		Expected		χ^2
	No.	Proportion	No.	Proportion	
A	17	0.500	15.79	0.4643	0.09
B	2	0.0882	3.45	0.1015	0.06
O	12	0.3529	13.10	0.3853	0.09
AB	2	0.0588	1.65	0.0486	0.07
					$\Sigma \chi^2 = 0.31$

DISCUSSION

It has been convincingly shown by experiments in mice that the immune responsiveness to dextran and to pneumococcal polysaccharide Type 14, respectively, is controlled by loci closely linked to *Ig* allotype loci (Blomberg *et al.*, 1972; Mäkelä *et al.*, 1980).

As mentioned in the introduction, several studies show that neonatal infections in man with GBS predominantly occurs in infants of mothers who have a low level of type-specific anti-carbohydrate IgG antibodies. Recently, it was found that these mothers have low

levels of antibodies against a variety of bacterial carbohydrate antigens (Christensen *et al.*, 1981). The data here presented on the abnormal distribution of *Gm* allotypes in these mothers fit well with an assumption that the IgG immune response to these bacterial carbohydrates is influenced by genes closely associated with the *Gm* loci. As is well-known, the *Gm* loci for the various subclasses of IgG are tightly linked (see Grubb, 1970). Many observations (Riesen *et al.*, 1976; Siber *et al.*, 1980; Young *et al.*, 1968, a.o.) indicate that IgG2 is the subclass predominantly involved in anti-carbohydrate response. It is indicated to investigate the *G2m* allotypes of these mothers.

As shown by the data of Pandey *et al.* (1979) and of de Vries *et al.* (1979) and of the present investigation, immune response genes in man are in linkage disequilibrium not only with the MHC complex but also to *Gm* loci.

Among their twenty-one mothers to infants with GBS-septicemia, Reid & Lloyd (1980) found six with blood group B, 28.6% compared to the distribution 10.6% in the population ($P < 0.05$). We were not able to confirm such a correlation in the present study.

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MOUSE-PROTECTIVE EFFECT OF RABBIT ANTI-R-PROTEIN ANTIBODIES
AGAINST GROUP B STREPTOCOCCI TYPE II CARRYING R-PROTEIN

Lack of Effect on Type III Carrying R-Protein

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R-protein was isolated by affinity chromatography from an alkaline extract of group B streptococci, type III. The identity of the protein was demonstrated by pepsin- and trypsin-treatment and in double diffusion-in-gel with antisera from other laboratories. A monospecific rabbit antiserum against the R-protein was studied in mouse protection tests, using three type II strains with R-protein and three without, and six type III strains, of which four carried R-protein. The serum protected animals from infection by type II strains carrying R-protein, but not against any of the other strains. The capacity of anti-R-protein serum to opsonize type II strains carrying R-protein was also demonstrated in phagocytosis experiments with mouse peritoneal macrophages. Studies with radiolabelled protein A and anti-R-protein antibodies showed that R-protein was localized on the surface of both type II and type III group B streptococci. Taken together, the results suggest that type II group B streptococci should be divided into two subtypes, II, R+ and II, R-.

Key words: group B streptococci; R-protein; type antigens; mouse protection; phagocytosis; radiolabelled protein A.

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(introduction)

Group B streptococci are causative organism in many serious neonatal infections (8, 11). The bacteria isolated from human sources usually belong to one of the serotypes Ia, Ib, Ic, II, or III (13, 28). More recently, a rarely occurring type IV was described (22). Ia, Ib, II and III are recognized by the specific carbohydrate antigens, whereas type Ic shares the carbohydrate antigen with type Ia, and the Ibc protein antigen with type Ib (28). Apart from these type antigens, the R and X antigens have been used for epidemiological characterization of group B streptococci (20, 21). Antigen R, which is trypsin-resistant and pepsin-sensitive, is closely related to the R-antigen in type 28 group A streptococci; this antigen has also been found in some strains of other serological groups, viz. C, G (18) and L (23). Wilkinson (26) found at least four immunologically distinct R-antigens in various combinations among certain strains of groups A, B and C. Antigen X, on the other hand, is relatively trypsin-labile and has been suggested to form a part of the antigenic structure of antigen R. Serologically, it is clearly distinguishable from antigen R (12).

As regards certain other bacterial species, it has been a tradition in the streptococcal field to base serotype nomenclature on mouse protection tests, i.e. a type antigen is so designated if specific antibodies against it will protect mice from infection with bacteria carrying the structure (14). Although the Ibc protein for instance can occur in type III group B streptococci (12, 28), the rationale for the designation type Ic was the specific mouse protection pattern of strains carrying Ia and Ibc antigens (14). On the other hand, type III group B streptococci have not been investigated in protection tests until recently (3), as it was long believed that these bacteria were avirulent for mice (1).

Hitherto, the R-antigen has been considered to be without importance for the virulence of group B streptococci, a view based on the observation that anti-R antibodies are not mouse-protective against type 28 group A streptococci (15). For this reason, the R-protein has not been considered to be a "true" type antigen in group B streptococci. The present work demonstrates, however, that rabbit anti-R antibodies protect mice from infection with type II group B streptococci carrying the antigen. On the other hand, no protection was obtained against type III carrying the R-protein. These findings call for a revision of the typing scheme for group B streptococci, or, alternatively, a revision of the basis for designation of antigens as type antigens in group B streptococci.

MATERIALS AND METHODS

Bacterial Strains and Extracts

The following reference strains of group B streptococci were used: types Ia (090), Ib (H36B), II (18RS21) and III (D136C). Six type II strains, three with R-protein (strains 101, 102, 103) and three without (strains 104, 105, 106), and six type III strains, four with R-protein (strains 107, 108, 109, 110) and two without (strains 111, 112) were selected from clinical isolates in our laboratory.

For detection of R-protein, alkaline and acid extracts of group B streptococci were prepared from overnight cultures at 37°C. in 300 ml Todd Hewitt broth. The bacteria were washed three times in saline, suspended in 2 - 3 ml saline and the pH raised to 10.0 with NaOH, alternatively lowered to 2.0 with HCl. The suspensions were then boiled on a waterbath for 10 min, the pH adjusted to 7.2 with HCl, alternatively NaOH and the tubes centrifuged. The resulting supernatants were adjusted to a final volume of 5 ml.

R-protein was purified from an extract prepared from 8 x 5 l overnight culture of a type III strain (No 107) in Todd Hewitt broth. The bacteria were harvested in a continuous action rotor in a Hi-spin 21 centrifuge (MSE, Sussex, England) at 9.000 r.p.m. Alkaline extract was prepared as described above, with the exception that the final volume of the extract was 150 ml.

Enzymatic Treatment of Bacteria and Extracts

Trypsinization was performed in phosphate-buffered saline (PBS, 0.12 M NaCl, 0.03 M phosphate, pH 7.2) at 37°C for 2 h, using trypsin (Sigma, St. Louis, MO, U.S.A.) at a final concentration of 5 mg/ml; the digestion was stopped with soybean trypsin inhibitor (Sigma), 10 mg/ml. Pepsin treatment was performed in saline, pH 3.0 (adjusted with HCl) at 37°C for 18 h, using the enzyme (2900 IU/ml; Sigma) at a final concentration of 5 mg/ml; the digestion was stopped by raising the pH to 7.4 with NaOH.

Rabbit Antisera

Antisera to the reference type strains and a clinical isolate of type III streptococci carrying R-protein (strain No 107) were obtained as described by Rotta et al. (24) for group A streptococcal antisera, with the exception that the rabbits were bled after only 3 weeks of immunization. Specific rabbit antiserum to R-protein was kindly supplied by Dr. N.E. Jensen, the State Veterinary Serum Laboratory, Ringsted, Denmark.

Antiserum to R-protein was prepared in one rabbit by immunization with 1 ml R-protein solution (see later), mixed with 1 ml complete Freund's adjuvant (Difco Lab., Detroit, Mich., U.S.A.) three times at one-week intervals. On each occasion, injections were given subcutaneously at two different sites in the neck.

IV

Separation of Group B Streptococcal Antigens by Affinity Chromatography

Antiserum to type III strain D136C, 15 ml diluted 1:4, was absorbed twice with pellets from overnight cultures of type II strain 18RS21 at 37°C in 500 ml Todd Hewitt broth. Antiserum to a clinical type III strain carrying R-protein (No 107) was absorbed in the same proportions with the type III strain D136C. The antisera were then separated on a Sephadex G200 column (Pharmacia, Uppsala, Sweden) using PBS as buffer. The second peak, containing IgG, was applied to a protein A-Sepharose 4B column (Pharmacia). After washing the column with PBS, IgG was eluted with 0.1 glycine buffer, pH 3.0. The IgG from the two antisera was then bound to two different Sepharose 4B columns (Pharmacia) following the recommendations of the manufacturer.

Alkaline extract of a type III strain containing R-protein (No 107) was applied on the column containing anti-III antibodies attached to Sepharose 4B. The fractions not adhering to the column during washings with PBS were pooled, concentrated on an UM10 filter (Amicon, Lexington, Mass., U.S.A.) and used for further isolation of R-protein. Type III antigen was eluted from the column by using 3 M KSCN, pH 7.4. The fractions containing R-protein were then applied on a column of anti-R antibodies attached to Sepharose 4B. After washings of the column with PBS, the R-antigen was eluted using 3 M KSCN in PBS, pH 7.4. Finally, the preparations of separated R-protein and type III antigen were concentrated and dialysed against PBS. The final volume corresponded to the volume of the crude extract first applied.

Double Diffusion-in-Gel and Crossed Immunoelectrophoresis

Double diffusion-in-gel was performed in 0.6 per cent agarose (EMC, Corp., Rockland, ME, U.S.A.) in 0.075 barbital buffer, pH 8.6, containing 2 mM calcium lactate. For crossed immunoelectrophoresis, 20 ml of this agarose was poured over a 205 x 110 mm glass plate and 10 mm broad slits each taking 5 µl extract were cut in the gel. After application of the extract a current of 250 V was applied for 45 min. A 5-mm broad slab was then cut out of the gel parallel with the direction of the current, covering the separated extract. This slab was placed edge-to-edge, at right angles to the projected direction of the current against agarose gel containing 0.5 per cent (vol/vol) antiserum to type III streptococci carrying R-protein, or 1.5 per cent antiserum to separated R-protein. A current of 250 V was applied for 45 min, and the plate dried. Precipitates were stained with Coomassie Brilliant Blue.

Detection of Group B Streptococcal Surface Antigens Using Antisera

Protein A from Staphylococcus aureus (Pharmacia) was radiolabelled with ¹²⁵I (19) and used to demonstrate group B streptococcal surface antigens, as described elsewhere (5). In brief, group B streptococci were grown overnight in 10 ml Todd Hewitt broth at 37°C and suspended in 1 ml PBS. Twenty-five µl serum was added to 200 µl of the suspension, the tube incubated at 37°C for 30 min and the bacteria washed with 2 ml PBS. Fifty

μ l radiolabelled protein A (0.2 μ g) was added, followed by a new washing. The radioactivity bound to the surface of the bacteria via the Fc part (9) was expressed as a percentage of the total amount of radioactivity added.

Phagocytosis Experiments

The capacity of mouse peritoneal macrophages to phagocytose group B streptococci opsonized with rabbit antisera and complement was studied using the method of Ginsburg et al. (10). In brief, 2.5×10^8 bacteria was incubated with 100 μ l antiserum diluted 1:10 and 10 μ l fresh guinea pig serum for 30 min at 37 °C. The bacteria were then washed and 10^7 opsonized bacteria added to 10^6 macrophages adhering to coverglasses in Petri dishes. After incubation at 37°C for 1 h, non-engulfed bacteria were washed away, and the macrophages "swollen" in a hypertonic solution, dried, and stained with Giemsa. The results were expressed as percentage phagocytosis, i.e. per cent of cells containing bacteria, and phagocytic index, i.e. mean number of bacteria per cell.

Mouse Protection Tests

These tests were performed as described previously (16). In brief, each animal was injected intraperitoneally with either 0.5 ml antiserum diluted 1:10, or 0.5 ml saline followed by 10^8 colony-forming units of the group B streptococci to be tested.

Statistical Method

Fischer's exact test was used (n.s. = not significant, i.e. $p > 0.05$).

RESULTS

Separation of R-Protein from Group B Streptococci Type III

R-protein and type III antigen from strain 107 were separated by affinity chromatography as described above. The type III antigen precipitated with antiserum to the type III reference strain in double diffusion-in-gel - but not with anti-Ia, anti-Ib, anti-II antisera or with antiserum to the purified R-protein. The latter serum, on the other hand, precipitated the R-protein, giving a line of non-identity with the type III antigen/anti-III precipitate (Fig. 1). Furthermore, anti-R-protein serum showed complete identity with an anti-R-protein serum (kindly supplied by Dr. N.E. Jensen) when the two antisera were tested against the R-protein in double diffusion-in-gel.

In crossed immunoelectrophoresis, the R-protein preparation gave one anodally located precipitate with the antiserum to the clinical type III isolate carrying R-protein, as well as with the antiserum to purified R-protein (Fig. 2). This precipitate could be completely abolished with pepsin; after trypsinization, the precipitate moved anodally (Fig. 2).

Antiserum to purified R-protein was tested in double diffusion-in-gel against acid as well as alkaline extracts of the reference strains and all clinical isolates. Precipitates were obtained only with strains containing R-protein and only a single precipitate was seen in these cases.

Demonstration of Mouse Protective Effect of Anti-II and Anti-III Sera for Homologous Type Strains

As expected, anti-II serum protected mice against infection with the clinical type II isolates, strains 101-106, when compared with control animals not given antiserum. Furthermore, the antiserum to the type III reference strain D136C gave protection against infection with all type III isolates, strains 107-112, used in the present investigation. On the other hand, anti-III serum did not protect against type II strains and vice versa (Table 1).

Protective Effect of Anti-R-Protein Serum for Type II Strains Carrying R-Protein

The antiserum against R-protein was tested for its capacity to protect mice challenged with type II strains. The serum protected against all ~~three~~ strains carrying R-protein, 101, 102 and 103, whereas no effect was seen against type II strains without R-protein (Table 2). In accordance with these findings, anti-R serum kindly supplied by Dr. N.E. Jensen protected mice against infection with strain 101, but not against strain 104.

Lack of Protective Effect of Anti-R-Protein Serum for Type III Strains Carrying R-Protein

The antiserum against R-protein did not protect mice challenged either with the four clinical type III isolates carrying R-protein (strains 107-110) or with the two strains without R-protein (strains 111 and 112).

Opsonization of Type II Strains Carrying R-Protein by Anti-R-Protein Serum in Phagocytosis Experiments with Mouse Peritoneal Macrophages

The antiserum against R-protein was tested for its capacity to opsonize type II group B streptococci with and without R-protein. The bacteria were incubated with antiserum and fresh guinea pig serum and the capacity of mouse peritoneal macrophages to phagocytose the bacteria was tested. Anti-R-protein elicited phagocytosis of R-protein-carrying bacteria in the same magnitude as shown by anti-II serum, whereas type II strains without R-protein were opsonized to a considerably lesser degree by anti-R-protein serum than by anti-II serum (Table 3).

Demonstration of R-Protein as A Surface Antigen in Some Group B Streptococci Types II and III

The clinical isolates, strains 101-112, were tested for binding of radiolabelled protein A after coating with anti-II, anti-

III and antiserum to R-protein. Coating of the type II strains with anti-II serum gave a binding of 72-85 per cent of the protein A added in the test, whereas anti-III resulted in less than 7 per cent binding for all type II strains. Anti-III serum resulted in binding between 74 and 88 per cent to the type III strains, in contrast to anti-II serum which gave an uptake of less than 6 per cent with the type III strains.

The antiserum to R-protein elicited a binding of 85-90 per cent for all strains carrying R-protein, i.e. strains 101-103 and 107-110, but less than 7 per cent protein was bound to the remaining clinical isolates 104-106 and 111 and 112, all without R-protein.

DISCUSSION

The results showed that it was possible to prepare an anti-serum monospecific for R-protein from group B streptococci without applying absorption procedures. The pepsin sensitivity of the preparation referred to here as R-protein, and the results with anti-R protein serum from another laboratory, left no doubt about the nature of the substance. The fact that the R-protein precipitate changed its electrophoretic mobility after trypsinization might indicate digestion of certain structures which were without influence on the antigenic characteristics of the molecule; in accordance, R-protein can be released from type 28 group A streptococci by trypsinization (15). Furthermore, our preparation of R-protein was kindly investigated by Dr. B. Perch (State Serum Institute, Denmark) who found that the preparation precipitated antiserum to type 28 group A streptococci in double diffusion-in-gel, identifying itself with R-protein from such a strain. The monospecific anti-R-protein serum gave definite protection of mice against all type II isolates containing R-protein, but not against type II isolates without R-protein or any of the type III isolates, irrespective of the presence of R-protein in these strains. The mouse protection experiments with antisera against the reference type II and III strains showed that the clinical isolates had been typed correctly. Furthermore, the results of phagocytosis experiments with mouse peritoneal macrophages confirmed that type II strains carrying R-protein were opsonized by anti-R-protein serum. All these results supported the conclusion that anti-R-protein afford protection to mice against type II strains carrying this antigen. According to classical definitions, type II group B streptococci can thus be divided into two subtypes, for which we suggest the names II, R+ and II, R-.

Anti-R-protein serum could not protect mice against infections with group B streptococci type III carrying R-protein. The experiments with radiolabelled protein A demonstrated that R-protein was localized on the surface of both type II and type III, excluding the possibility that the type III antigen covered the R-protein on type III group B streptococci. Several

investigators have reported a significant role for complement in the opsonization of group B streptococci type III (2, 25). Sialic acid constitutes an important part of the type III antigen and is thought to prevent activation of the alternative pathway (7). Further studies are needed to elucidate the possibility that sialic acid of type III antigen might prevent opsonization of group B streptococci type III via anti-R-protein antibodies. It is also possible that anti-R-protein antibodies could promote opsonization if small amounts of anti-III antibodies were present; this theory is currently under investigation in our laboratory.

The results described here did not justify speculations concerning the possible importance of the R-protein as a virulence factor in human infections. However, there are several indications that human beings are often exposed to R-protein. Studies in our district have demonstrated that R-protein occurs in 51 per cent of all type II strains and in 86 per cent of all type III strains isolated from unselected clinical specimens (17). About 16 per cent of the parturients at our hospital are urogenital carriers of group B streptococci (6) and types II and III predominate among the strains isolated from this site (6). R-protein is also found among group A, C, and G streptococci (18); although differences may exist between R-protein from different bacteria; these substances seem to cross-react extensively (26). Furthermore, groups B and L streptococci carrying R-protein are frequently isolated from cows with mastitis, which indicates that R-protein might be present in minute amounts in consumer's milk.

In conclusion, the present study has demonstrated that antibodies against R-protein protect mice from challenge with type II group B streptococci carrying this protein, but not against type III R-protein-positive group B streptococci. It is suggested that the type II group B streptococci can be subdivided into two subtypes, II, R+ and II, R-. The significance of the findings for human immunity against group B streptococci remains to be studied.

TABLE 1. Mouse Protective Effect of Rabbit Anti-II and Anti-III Sera for Homologous Type Strains^a

Strain no.	Type	Number of mice killed/injected after		
		saline	anti-II	anti-III
101	II, R+ ^b	7/10	0/10*	7/10
102	II, R+	7/10	0/10*	7/10
103	II, R+	6/10	0/10*	6/10
104	II, R- ^c	10/10	0/10*	10/10
105	II, R-	10/10	0/10*	9/10
106	II, R-	10/10	0/10*	9/10
107	III, R+	10/10	10/10	2/10*
108	III, R+	8/10	8/10	1/10*
109	III, R+	9/10	9/10	1/10*
110	III, R+	9/10	10/10	3/10*
111	III, R-	10/10	10/10	2/10*
112	III, R-	8/10	8/10	2/10*

^aEach animals was given 10^8 CFU of the group B streptococcal strain to be tested and 0.5 ml antiserum diluted 1:10 or 0.5 ml saline.

^bStrain carrying R-protein.

^cStrain without R-protein.

* Significantly fewer animals died as compared with the control group given saline ($p < 0.05$).

Table 2. Mouse Protective Effect of Rabbit Anti-R-Protein Serum for Type II Strains Carrying R-Protein^a

Strain no.	Type	Number of mice killed/injected after		p-value
		saline	anti-R-Protein	
101	II, R+ ^b	9/10	2/10	0.003
102	II, R+	9/10	3/10	0.01
103	II, R+	9/10	0/10	0.00006
104	II, R- ^c	8/10	8/10	n.s.
105	II, R-	7/10	6/10	n.s.
106	II, R-	10/10	8/10	n.s.

^a Each animal was injected with 10^8 CFU of the group B streptococcal strain to be tested and either 0.5 ml antiserum diluted 1:10 or 0.5 ml saline. The antiserum was raised against R-protein isolated from a type III strain.

^b Strain carrying R-protein.

^c Strain without R-protein.

TABLE 3. Opsonization of Type II Strains Carrying R-Protein by Rabbit Anti-R-Protein Serum in Phagocytosis Experiments with Mouse Peritoneal Macrophages^a

Strain no.	Type	Antiserum	Percentage phagocytosis	Phagocytic index
102	II, R+ ^b	anti-II	50	11.0
102	II, R+	anti-R-protein	50	5.5
103	II, R+	anti-II	53	11.8
103	II, R+	anti-R-protein	75	12.0
104	II, R- ^c	anti-II	55	10.5
104	II, R-	anti-R-protein	20	1.1
106	II, R-	anti-II	45	9.6
106	II, R-	anti-R-protein	3	0.1

^a The bacteria were opsonized with antiserum and fresh guinea pig serum. Controls with saline and guinea pig serum gave no phagocytosis.

^b Strain carrying R-protein.

^c Strain without R-protein.

LEGENDS TO FIGURES

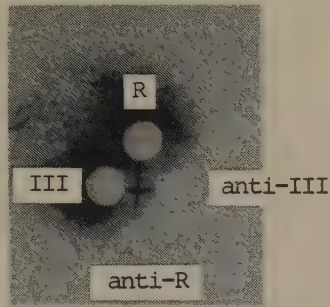


Fig.1. Non-identity between R-protein and type III antigen from group B streptococci. Well labelled R contained R-protein anti-R contained anti-R-protein serum, III contained type III antigen and anti-III contained anti-type III serum. The double diffusion-in-gel was stained with Coomassie Brilliant Blue.

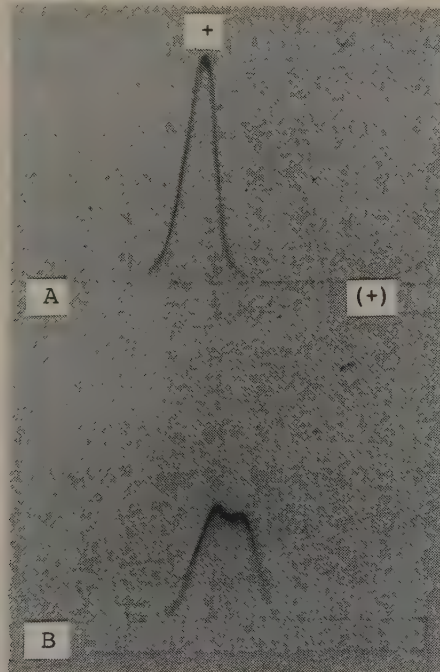


Fig.2. Crossed immunoelectrophoresis of R-protein isolated from type III strain 107 using antiserum to strain 107. (+) indicates the anode in the first dimension separation; +, the anode in the second dimension; A, the application well for R-protein; B, application well for a mixture of untreated R-protein and trypsinized R-protein (equal volumes).

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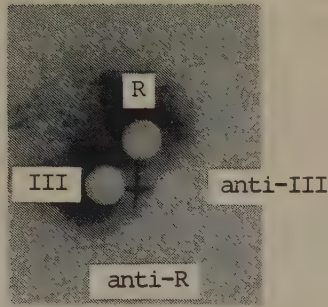


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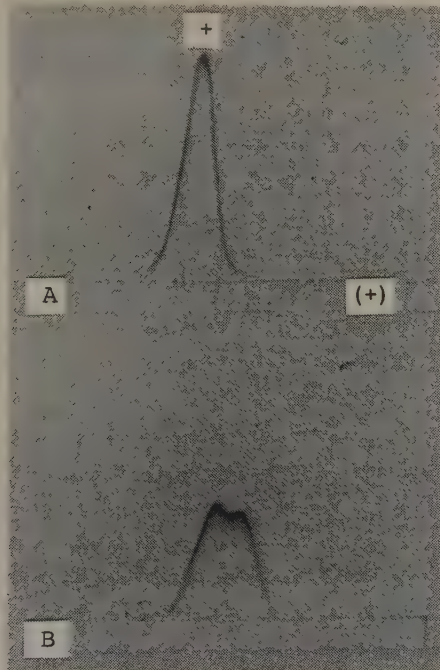


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THE OCCURRENCE OF R-PROTEIN AMONG ISOLATES OF GROUP B
STREPTOCOCCI FROM HUMAN SOURCES

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Lindén, V., Christensen, K.K. & Christensen P. The occurrence of R-protein among isolates of group B streptococci from human sources. Acta path. microbiol immunol. scand. Sect.B,

A total of 241 clinical isolates of group B streptococci were tested for presence of R-protein by applying the double diffusion-in-gel technique on streptococcal extracts against monospecific anti-R protein serum, radiolabelled protein A and anti-R protein serum or S. aureus; Cowan I coated with antibodies against group A streptococci, T-type 28. The first two methods proved more sensitive than the latter. R-protein was detected in 51 % of 43 type II strains and in 87 % of 102 type III strains, whereas none of 79 type Ia and none of 17 type Ib strains contained R-protein. Among 30 isolates from blood and cerebrospinal fluid of infants with neonatal infections, R-protein was detected in 43 %, one of two type II strains and 12/14 type III strains. Tests of 37 paired strains from infant/mother pairs showed concordant results with respect to presence or absence of R-protein in the isolates.

Key words: group B streptococci; typing; R-protein; neonatal infections.

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(introduction)

Group B streptococci cause serious infections in the neonatal period (1,4,6). The bacteria isolated from human sources usually belong to one of the serotypes Ia, Ib, Ic, II or III (11, 20) or, more seldom, type IV (18). Types Ia, Ib, II and III are recognized by specific carbohydrate antigens and type Ic shares the Ia carbohydrate antigen with type Ia and the Ibc protein with type Ib (11, 20). Apart from these type antigens, the R and X proteins have been used for epidemiological characterization of group B streptococci, especially strains isolated from bovine sources (7, 8, 16, 17). Antigen X is very seldom found in human strains (7). R-protein of group B streptococci is closely related to the R-antigens in group A, C and G streptococci (12, 14). Wilkinson (19) found at least four immunologically distinct R-antigens present among strains of groups A, B and C.

For many years it was believed that R-protein was of no importance for the virulence of group B streptococci, (15) a view based on the observation that anti-R antibodies are incapable of protecting mice against infection by group A streptococci type 28 (12). Recently, we demonstrated that anti-R antibodies are in fact capable of protecting mice from infection by type II group B streptococci carrying this antigen, but not against type II without R-protein or against type III, irrespective of the presence of R-protein (13). These studies indicated that R-protein might be of importance for the virulence of type II group B streptococci. We therefore found it of interest to study the occurrence of R-protein among human group B streptococcal strains.

MATERIALS AND METHODS

Bacterial Strains and Extracts

Eight strains of group B streptococci belonging to type Ia, six to type Ib, two to type II and 10 to type III were isolated in Lund and Stockholm from blood or cerebrospinal fluid (CSF) of infants with neonatal group B streptococcal infections. In 12 cases of infections with types II and III, strains were also available from the maternal vagina.

Paired strains, from infants (throat, ear or umbilical, specimens) and mothers (urogenital tract), colonized but not infected, belonging to types II (10 pairs) and III (15 pairs) were also studied.

The remaining strains used in the investigation, 71 belonging to type Ia, 11 to type Ib, 19 to type II and 48 to type III, were isolated from the urogenital tract of healthy parturients. These strains and the paired strains from colonized infants and mothers were isolated in Lund.

Strains belonging to type Ic were not included in the investigation because of the rarity of this type in our district.

All strains were typed by using co-agglutination (5).

Extracts of the strains were prepared from overnight cultures at 37 °C in 300 ml Todd-Hewitt broth. The bacteria were washed three times in saline, suspended in 2-3 ml saline and the pH raised to 10.0 with NaOH. The suspensions were then boiled on a water bath for 10 min, the pH adjusted to 7.2 with HCl and the tubes centrifuged. The resulting supernatants were adjusted to a final volume of 5 ml.

Detection of R-protein

R-protein was detected by using three different methods:

- (a) immunodiffusion : a monospecific antiserum to R-protein from group B streptococci type III was used in double diffusion-in-gel against alkaline extract of the strain to be tested (13);
- (b) co-agglutination using Staphylococcus aureus, Cowan I coated with a commercial anti-T28 serum (Chemapol, Prague) as described (3); and (c) radiolabelled protein A from Staphylococcus aureus (Pharmacia, Uppsala) and monospecific anti-R-protein serum as described elsewhere (13).

RESULTS

Distribution of R-Protein among the Total Material of Clinical Isolates of Group B Streptococci

A total of 241 clinical isolates of group B streptococci was tested for presence of R-protein by using immunodiffusion and radiolabelled protein A. Concordant results were obtained with the two methods. Not a single strain among 79 type Ia isolates or 17 type Ib strains carried R-protein. On the other hand, 51 per cent of 43 type II strains and 87 per cent of 102 type III strains carried R-protein (Table 1).

Co-agglutination with staphylococci coated with anti-T28 serum revealed the presence of R-protein in 21/22 type II and 80/89 type III strains shown to contain R-protein by the other two methods, but detected no further R-proteincontaining strains. The following results apply to tests with immunodiffusion and radiolabelled protein A.

Occurrence of R-Protein among Group B Streptococci Isolated from Infants with Neonatal Group B Streptococcal Septicemia/Meningitis

A total of 30 strains isolated from cases of group B streptococci septicemia/meningitis were tested for presence of R-protein. The protein was detected in 13 strains (43 per cent), one of two type II strains and 12/14 type III (Table 2).

Occurrence of R-Protein in Group B Streptococci Types II and III Isolated from Infant/Mother-Pairs

Strains of type II and type III group B streptococci isola-

ted from 37 infants and their respective mothers were tested for R-protein. Concordant results were obtained in all cases from the maternal vs. and infants strains with respect to presence/absence of R-protein. R-protein was detected in 30/37 paired strains of types II or III (Table 3).

DISCUSSION

Hitherto, few reports have been published concerning the distribution of R-protein in clinical isolates of group B streptococci from human sources. The finding in the present study that 87 per cent of type III strains contained R-protein confirmed the report by Jensen (9) who found R-protein in 93 per cent of his type III isolates from the female urogenital tract, whereas Jelínková reported only 16 per cent of human type III strains as carrying R-protein (7). On the other hand, Jelínková (7) reported 56 per cent of human type II isolates as carrying R-protein which correlates closely with our frequency of 51 per cent but somewhat higher than the 30 per cent in Jensens study (9). We found no type Ia or Ib strains with R-protein, which does agree with Jensen's report (9). Jelínková (7) reported Rprotein as occurring in 10 per cent of type Ia strains and in about 1 per cent of type Ib strains.

To our knowledge, the present report concerns the first systematic study of the occurrence of R-protein among strains isolated from blood or CSF of neonates. R-protein was found in 12/14 type III strains, (86 per cent) corresponding closely with the frequency in the whole material of type III streptococci. It is difficult to assess the clinical importance, if any, of R-protein in type III strains. We were not able to demonstrate any mouse-protective effect of anti-R-protein antibodies against experimental type III infections. On the other hand, such antibodies do protect mice from infection by type II strains carrying R-protein (13) which might contribute to the low frequency of type II strains in neonatal meningitis also reflected in the present report, provided that anti-R antibodies are also protective in the human.

R-protein is generally considered to be "unstable" (15); the meaning of this characterization as well as the scientific background to such a statement are unclear. The present results demonstrated that R-protein is in fact stable as an epidemiological marker in the sense that identical results were obtained with paired strains from mother and infant.

In the whole material of group B streptococci studied here, 46 per cent contained R-protein. Assuming a urogenital carrier rate of 16 per cent among all women (2), about 8 per cent of the fertile female population is constantly exposed to R-protein. Cross-reactive proteins are also present in group A streptococci types 3 (10) and 28 (12) and in β -hemolytic streptococci of groups C and G (14). Furthermore, R-proteins are commonly found in bovine group B streptococcal strains (7), indicating that minute amounts of R-protein might be present in consumer milk.

These facts, taken together suggest that man is heavily exposed to R-proteins and call for further studies on human antibody response to R-protein. Such investigations are currently being performed in our laboratory.

Table 1. Occurrence of R-Protein among Group B Streptococci Types Ia, Ib, II and III in the whole Material of Strains

R-protein	No. (per cent of group B streptococci types			
	Ia	Ib	II	III
Present	0	0	22(51)	89(87)
Absent	79(100)	17(100)	21(49)	13(13)
Total no.	79	17	43	102

Table 2. Occurrence of R-Protein among 30 Group B Streptococci Strains Isolated from Infants with Septicemia/Meningitis

Type of group B streptococci	R-protein	No. of strains
Ia	present	0
	absent	8
Ib	present	0
	absent	6
II	present	1
	absent	1
III	present	12
	absent	2

Table 3. Occurrence of R-Protein in Group B Streptococci Types II and III Isolated from Infants and Their Mothers

Clinical diagnosis of infants	Type of group B streptococci isolated	No. of infant/mother pairs with			
		R-protein present in both infant- and maternal strain	R-protein absent from both infant and maternal strain	Diverging results of R-protein test	
Healthy	II	6	4	0	
Healthy	III	14	1	0	
Septicemia/meningitis	II	1	1	0	
Septicemia/meningitis	III	9	1	0	
Total no.		30	7	0	

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Correlation between Low Levels of Maternal IgG Antibodies to R-Protein and Neonatal Septicemia with Group B Streptococci Carrying R-Protein

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Abstract. A method was designed for quantitation of serum antibodies to R-protein in group B streptococci (GBS). Four sera from mothers of infants with neonatal septicemia caused by type II, R+ GBS showed a binding range from 400 to 740 counts per minute (cpm) in contrast to a range of 690 to 1220 cpm in five parturients carrying type II, R+ strains in the urogenital tract giving birth to healthy infants ($p < 0.05$). The range of antibodies in sera from 8 mothers to infants infected with GBS type III, R+ was 370 to 750 cpm whereas the range in sera from 10 carriers of type III, R+ giving birth to healthy infants varied between 510 and 1280 cpm ($p < 0.01$). These findings indicate that R-protein is an important virulence factor in neonatal GBS septicemia/meningitis.

Introduction

In 1976, Baker and Kasper (1) reported that mothers of infants with neonatal GBS type III septicemia are deficient in antibodies against type III carbohydrate antigen. The finding was later confirmed and extended to types Ia, Ib and II by several authors (4, 8, 19). It has also been shown that the protective maternal antibodies are of IgG class (4, 8, 19). Previous studies indicated that mothers of GBS-infected infants have low levels of IgG antibodies against a number of bacterial carbohydrate antigens (5) and that this deficiency probably is genetically based (7).

In contrast to carbohydrate antigens, little is known about the role of bacterial protein antigens in neonatal GBS infections. However, in vitro studies indicate that GBS protein antigens might exert an antiphagocytic effect (18). Furthermore, it is well established that antibodies to the Ibc-protein, a type antigen in Ib and Ic GBS, can protect mice from infections with strains carrying this antigen (11). Another protein antigen, the R-protein, was until recently considered to be without importance for the virulence of GBS (12). However, we were able to demonstrate that rabbit anti-R serum protected mice from infection with type II GBS carrying R-protein but not against type III GBS with R-protein (13).

R-protein is widespread among human GBS isolates. In our district 51% of type II and 87% of type III GBS strains carry R-protein whereas the antigen is not present among types Ia and Ib (14). We found it therefore interesting to study the role of maternal anti-R antibodies in human neonatal GBS infection. Our findings indicate that R-protein in fact is an important virulence factor in neonatal septicemia caused by GBS.

Materials and Methods

Patients

Study group. Sera from 16 mothers of infants who contracted GBS neonatal septicemia and/or meningitis and from 1 patient who experienced intrauterine fetal death due to GBS, were investigated. The 16 infants contracted their infection within 48 h of life. GBS were isolated at sterile autopsy (2) from all inner organs of the antenatally dead fetus and from blood and/or cerebrospinal fluid of the infected infants. All mothers were urogenital carriers of the strain infecting their infants. The infecting types were: type II, R-positive (R+), 4 patients; type II, R-negative (R-), 3 patients; type III, R+, 8 patients; and type III, R-, 2 patients. Sera were obtained from the mothers within 1 month after delivery. 15 of these sera have previously been studied for antibodies to GBS-type antigens (5, 7).

Control group. The control group consisted of 26 parturients who were urogenital carriers of GBS; their

infants showed no signs of infection during the neonatal period. Sera were obtained from the mothers within 1 month after delivery. The isolated GBS types were: type II, R+, 5 parturients; type II, R-, 8 parturients; type III, R+, 10 parturients; and type III, R-, 3 parturients.

Serological grouping and typing of GBS.

The strains were grouped and typed as previously described (3). R-protein was demonstrated in double diffusion-in-gel using a specific antiserum (13).

Rabbit Antisera

Antisera were raised as previously described (3) against the following reference strains of GBS: types Ia (090), Ib (H36B), II (18RS21) and III (D136C). Furthermore a previously described antiserum to isolated R-protein was also studied (13). Rabbit antiserum to T-type 28 group A streptococci was purchased from Chemapol, Prague, Czechoslovakia. Specific rabbit anti-human IgG3 was kindly supplied by Dr A. Grubb, Department of Clinical Chemistry, Allmänna Sjukhuset, Malmö, Sweden.

Detection of Antibodies to R-Protein

Antibodies against R-protein of GBS were quantitated by the use of radiolabelled protein A (4). Protein A from Staphylococcus aureus (Pharmacia, Uppsala, Sweden) was radiolabelled with ^{125}I (15). Standard suspensions of the GBS type III reference strain D136C, which lack R-protein, of a type III, R+ clinical isolate (strain 107, see ref 13), of a type II, R+ and a type II, R-, clinical isolates, were prepared as previously described (6).

Unless otherwise indicated, the serum to test, 0.5 ml was diluted with 1 ml phosphate buffered saline (PBS, 0.12 M NaCl, 0.05 M phosphate, pH 7.2) and added to the sediment from a 10 ml standard suspension of strain D136C spun down at 3000 g for 15 min. After suspension of the bacteria in the serum, and incubation at 37°C for 30 min, the tubes were centrifuged and the supernatant tested for antibodies to surface antigens of strain 107 as follows: (a) 200 μl PBS containing 10 mg/l of human serum albumin (AB Kabi, Stockholm, Sweden) was added to 11 x 70 mm polystyrene tubes (A/S NUNC, Roskilde, Denmark) followed by 200 μl standard suspension of strain 107; (b) addition of 100 μl of the absorbed serum and incubation at 37°C for 30 min; (c) 2 ml PBS containing 0.1 % (vol/vol) Tween 20 (AB Kebo, Stockholm, Sweden) was then added, followed by centrifugation at 3000 g for 30 min and suspension of the bacterial sediment in 200 μl PBS containing HSA; (d) addition of 50 μl radiolabelled protein A (0.5 μg) and incubation at 22°C for 10 min; (e) addition of 2 ml PBS containing Tween 20 and centrifugation of the tubes as described above. The radioactivity in the sediment was then counted and expressed in counts per minute (cpm).

In some experiments, the serum to test was absorbed with a type II, R+ or a type II, R- strain instead of strain D136C.

Experiments were also performed to study the possibility that anti-R-protein antibodies belonging to subclass IgG3 was present in the human sera tested, since protein A does not

bind to this subclass (10). In these tests, 25 μ l anti-human IgG was added after step (c) above, the tube incubated at 37°C for 30 min, the bacteria washed with PBS-Tween 20 and the experiment continued with step (d) as described above. In this way IgG3 antibodies would bind rabbit IgG reacting with the protein A.

All test were performed in duplicates.

Statistical Analysis .

Student's t -test was used throughout the study.

Results

Investigation of the Specificity of the Test for Quantitation of Anti-R-Protein Antibodies .

Various rabbit antisera were tested unabsorbed for antibodies to surface antigens in strain 107 (type III, R+) using radiolabelled protein A. Undiluted antiserum to isolated R-protein gave a binding of 7500 cpm protein A, decreasing in uptake with increasing dilution of the serum. Preimmune serum from the same rabbit gave only 150 cpm, and the binding was unrelated to dilution (fig.1). Undiluted anti-Ia, -Ib and -II sera resulted in a binding of 1500 cpm, and this binding decreased to 200 cpm by absorption with strain D136C.

R-protein in group A streptococci type 28 is known to cross-react with R-protein in GBS (12). As expected, undiluted anti-T28 serum gave a high uptake of protein A, 7250 cpm, and the uptake decreased with increasing dilution of the serum (fig. 1.).

The antiserum to isolated R-protein was absorbed with type II, R+ and type II, R- GBS. A slightly diminished uptake was registered after absorption with type II, R-strain while the binding of radioactivity decreased substantially after absorption with II, R+ GBS (fig.1.).

A number of human sera were tested for antibodies against R-protein using the absorption step with strain D136C (III, R-) before measuring antibodies against strain 107 (III, R+). Among these sera, two specimens were selected for studies of the specificity of the test. Serum A gave an uptake of 2300 cpm and serum B a binding of 410 cpm (fig.2.). The radioactivity obtained with serum A diminished with increasing dilution and this serum could be diluted 1:96 before a level of 410 was obtained. Absorption of serum A with type II, R- GBS did not result in any diminishing of the binding of protein A to strain 107 whereas the binding decreased from 2300 cpm to 590 by absorption with type II, R+ GBS (fig. 2).

As mentioned, all test were performed in duplicates; the difference did not in any case exceed 10% of the mean of the two counts obtained.

Quantitation of Antibodies to R-Protein in Sera of Mothers to Infants with GBS-Septicemia; Comparison to Sera from GBS-Carriers Giving Birth to Healthy Infants .

Sera from mothers of infants infected with type II, R+ GBS were compared to sera from parturients who were urogenital carriers of this type and gave birth to healthy

infants. The binding obtained with the sera from the study group varied between 400 and 740 cpm whereas the controls ranged from 690 to 1220 cpm. The uptake of protein A in the study group sera was significantly lower than in the sera from the controls ($p < 0.05$) (fig. 3).

Similar results were obtained concerning mothers of infants infected with type III, R+ GBS and controls carrying such strains. The range of protein A uptake in sera from the study group was 370 to 750 cpm whereas the sera from the controls varied between 510 to 1280 cpm ($p < 0.01$) (fig. 3.).

No differences were found in anti-R-protein levels between study group and controls exposed to type II, R- GBS, or between study group and controls exposed to type III, R- strains (fig. 3.). Within the study group, there was no statistically significant difference between mothers of infants infected with type II, R+ and type II, R- or between types III, R+ and III, R-. However, when all mothers of infants infected with R+ strains were compared to mothers of R- infected infants, irrespective of type, the "R- group" showed significantly higher antibody levels ($p < 0.05$).

There was no significant difference between the carriers of types II, R+ and II, R-, or between the carriers of types III, R+ and III, R-.

Ten randomly selected sera among the patients and the controls were absorbed with type II, R+ GBS. In all sera the uptake of radiolabelled protein A decreased to below 200 cpm. In contrast, absorption with type II, R- strain had no such effect.

Anti-human IgG3 was added to the test system (see Materials and Methods) to test the possibility that some of the sera might contain anti-R antibodies belonging to the human IgG3 subclass. No change was observed in the binding of protein A for 10 randomly selected sera.

Discussion

A method was designed for quantitation of IgG antibodies to R-protein based on absorption of the sera with a type III, R- strain and quantitation of antibodies in the absorbed sera to a type III, R+ strain (No. 107) by the use of radiolabelled protein A. Experiments with unabsorbed rabbit antisera to various GBS types demonstrated that only small amounts of cross-reactive antibodies against GBS antigens interfered with the test; these antibodies were removed by absorption with strain D136C (type III, R-). Furthermore it was found that a type II, R+ strain could remove the antibodies binding to strain 107 from rabbit antisera as well as from human sera, whereas a type II, R- strain only had limited effect (fig. 1 and fig. 2). These findings indicated a sufficient specificity of the method for measuring antibodies against the R-protein of GBS.

Previous studies have demonstrated that mothers of infants with GBS septicemia are deficient in IgG antibodies

against the type-specific carbohydrate antigen of the strains infecting the respective infants (4, 5, 8, 19). Quantitation of IgG antibodies to GBS II and III demonstrated that the sera from mothers of GBS infected infants studied in the present work were no exceptions from this rule (unpublished observation). Our previous studies indicate that these mothers are poor responders to bacterial carbohydrate antigens in general (5) and that this deficiency is genetically based (7). On the other hand, their serum levels of IgM antibodies to carbohydrate antigens do not differ from or are higher than the serum levels of parturient GBS carriers giving birth to healthy infants (5). Purified bacterial capsular polysaccharides induce an antibody response pattern that has been characterized as "thymic-independent" since T-lymphocytes (helper cells) do not participate in the cellular response leading to antibody production (17). The present results showed that mothers of GBS-infected infants do not have low levels to R-protein in general, i.e. mothers of infants infected with R- strains did not deviate from the controls concerning anti-R antibodies. Since protein antigens commonly are characterized as "thymus-dependent" it is indicated that the immunological deficiency of mothers of GBS-infected infants seems to be related mainly to the immune response against bacterial carbohydrate antigens, i.e. "thymus-independent" antigens.

The present findings corroborate previous results demonstrating that R-protein is of importance for the virulence of GBS (13, 14). Thus, mothers of infants infected with type II, R+ strains and type III, R+ strains showed significantly lower levels than parturients who were urogenital carriers of the same strains but gave birth to healthy infants. In previous work (13) it was found that anti-R-protein antibodies protected mice against challenge with type II, R+ GBS but not against type III, R+ GBS. The present findings encourage further studies of the possible mouse-protective effect of anti-R antibodies against type III, R+ GBS; the possibility that anti-R antibodies might be protective in the presence of small amounts of antibodies to the type III carbohydrate antigen should be elucidated.

The results did not allow any conclusions about the possible benefit of vaccination of women with R-protein. The finding that mothers of infants infected with type II, R- and type III, R- GBS showed significantly higher levels of anti-R antibodies than mothers of infants infected with R+ strains might indicate that the presence of anti-R antibodies in the pregnant women might promote selection of variants which are R-.

Furthermore, the experiments with anti-IgG3 showed that anti-R antibodies belong to one or more of the IgG subclasses 1, 2 and 4. This is interesting since antibodies to M-associated protein mainly are of IgG3 subclass (16); R-protein is similar to streptococcal M protein in its physicochemical properties (9).

In conclusion, the present study has shown that mothers

of infants infected with R+ GBS have low levels of antibodies to GBS carrying R-protein. These results, together with the results from previous mouse-protection experiments (13) clearly indicate that R-protein is of significance for the virulence of GBS.

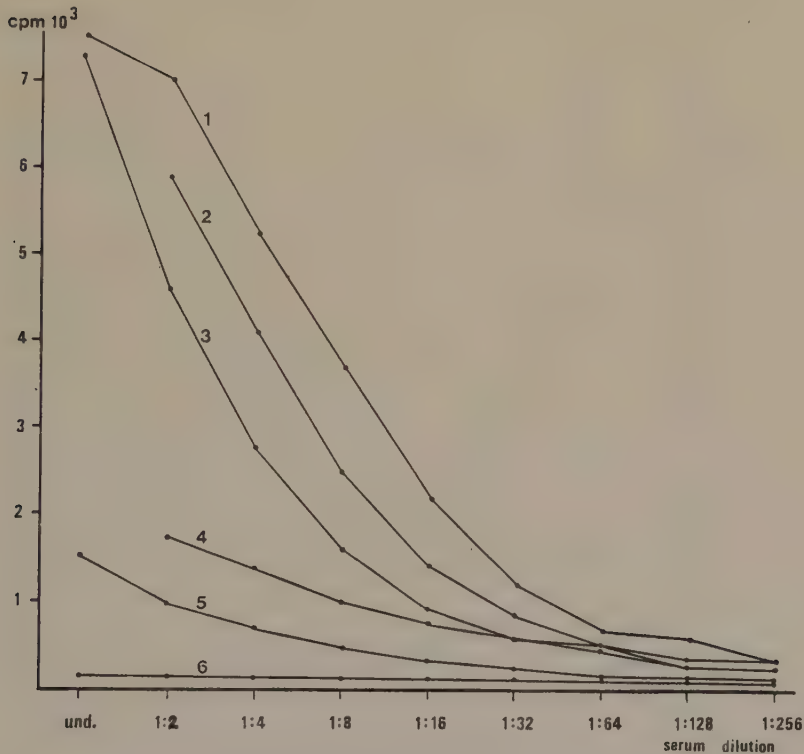


Fig.1. Quantitation of antibodies to strain 107 (type III, R+ GBS) in rabbit antisera to various streptococcal strains and to isolated R-protein. Abscissa: dilution of serum tested; ordinate: binding of radiolabelled protein A in cpm. 1 = unabsorbed antiserum to isolated R-protein; 2 = serum 1 absorbed with type II, R- GBS; 3 = unabsorbed antiserum to group A streptococci T-type 28; 4 = serum 1 absorbed with type II, R+ GBS; 5 = unabsorbed antiserum to type Ia GBS (anti-Ib and anti-II gave similar results); 6 = unabsorbed preimmune serum from the rabbit later immunized with isolated R-protein.

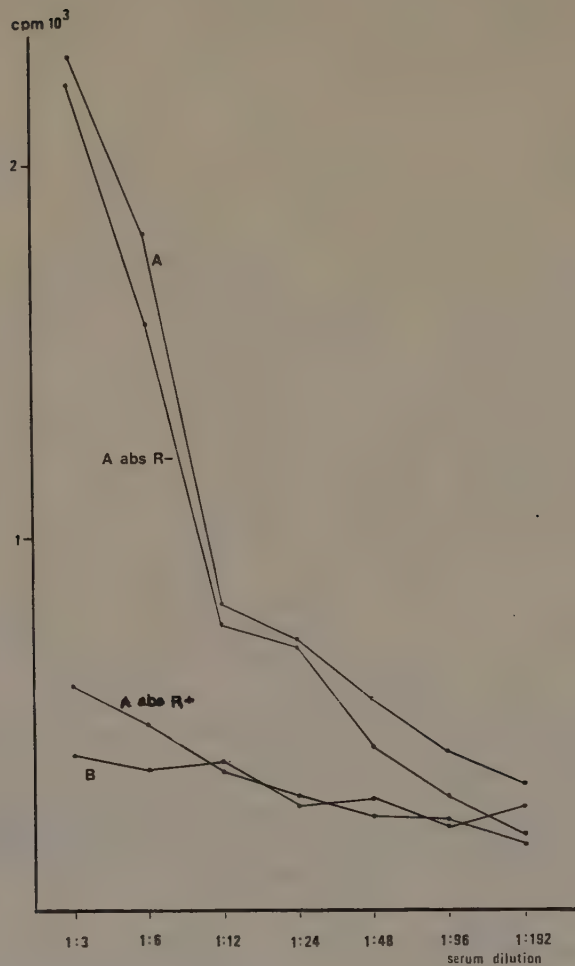


Fig. 2. Quantitation of antibodies to R-protein in human sera. The sera were absorbed with strain D136C (type III, R-) and antibodies against strain 107 (type III, R+) then quantitated using radiolabelled protein A. Abscissa: dilution of serum tested; ordinate: binding of radiolabelled protein A in cpm. A = serum A; B = serum B. A abs R+ = serum A absorbed with type II, R+ GBS instead of strain D136C; A abs R- = serum A absorbed with type II, R- GBS instead of strain D136C.

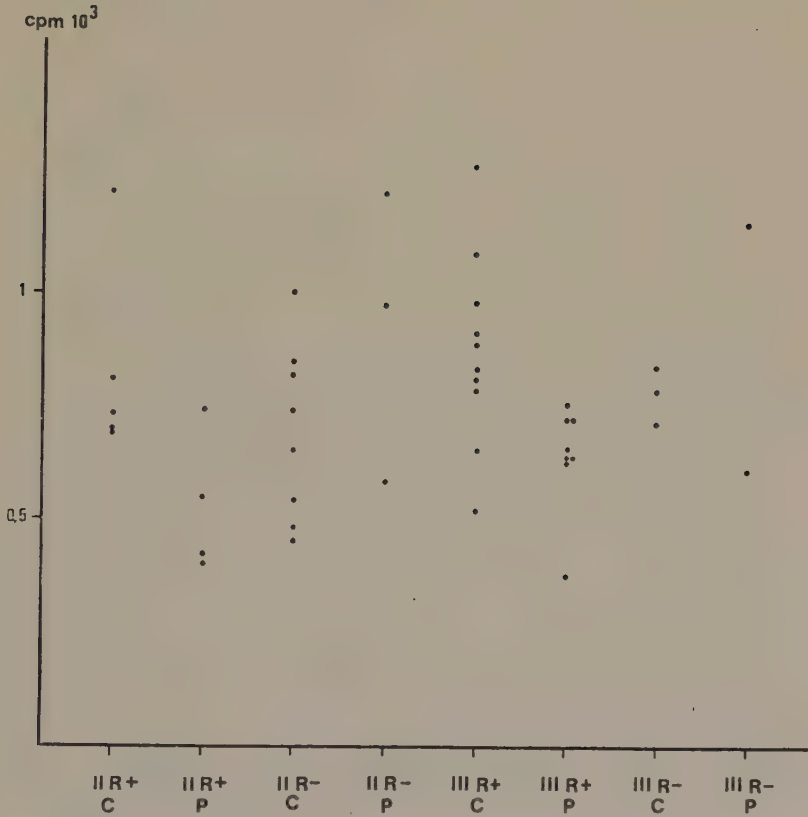


Fig. 3. Quantitation of antibodies to R-protein in sera from mothers to infants with GBS septicemia/meningitis and parturient carriers of GBS giving birth to healthy infants. Ordinate gives the serum levels of anti-R antibodies in cpm. C II, R+ = carriers of GBS type II, R+ giving birth to healthy infants; P II, R+ = mothers of infants infected with GBS type II, R+; etc.

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Low Levels of Antibodies to Surface Antigens of Group B Streptococci in Commercial IgG Preparations

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Abstract. 53 different batches of commercial IgG were tested for antibodies to group B streptococci (GBS) types Ia, Ib, II and III. The levels of antibodies varied widely. Comparison of the anti-GBS antibody levels in these preparations with those found in normal blood donor sera showed that the commercial IgG contained approximately 2.6 times less type-specific antibodies. We conclude that the commercial IgG now available is not optimal for passive immunization against GBS infections in neonates.

Introduction

Group B streptococci (GBS) cause serious infections in newborns [1]. Maternal anti-GBS antibodies, supplied through the placenta, seem to protect against such infections [3, 5, 9, 15]. On this background, it has been suggested to vaccinate women with GBS antigens, i.e., with the type-specific carbohydrate antigens [4]. However, recent reports indicate that individuals with low prevaccination levels of antibodies against bacterial carbohydrate antigens respond poorly to immunization with such antigens [14]. Our studies indicate that mothers to GBS-infected infants may be poor responders to bacterial carbohydrate antigens on a genetical basis [6, 8]. Another possibility might be to protect the infant at risk by passive immunization of the mother with IgG antibodies against GBS antigens. In order to elucidate this possibility we have tested various commercial IgG preparations for such antibodies.

Materials and Methods

Sera from 50 different healthy human blood donors were used. 53 batches of IgG, manufactured 1966–1979 were kindly supplied by Dr. H.D. Schlumberger, Bayer Pharma-Forschungszentrum, Wuppertal, FRG. The samples represented concentrations from 50 to 165 g/l. All were diluted to 17 g/l in phosphate-buffered saline (PBS, 0.15 M NaCl, 0.05 M phosphate, pH 7.2).

Human IgG was quantitated by electroimmunoassay [11].

Quantitation of antibodies against GBS surface antigens of types Ia, Ib, II and III was performed essentially as described previously [5, 6]. In brief, preparations to be quantitated for antibodies against types Ia, Ib and III were absorbed with type II, while absorption was performed with type III GBS for determination of anti-II. The absorbed preparation was then added to a suspension of GBS of the type against which antibodies should be quantitated, and the amount of IgG bound to the bacteria determined by the use of radiolabelled protein A of *Staphylococcus aureus*. The results were expressed as the radioactivity bound to the bacterial sediment, in counts per minute.

The commercial IgG preparations were centrifuged at 50,000 g for 30 min before test, in order to remove aggregates.

Results

The 53 batches of commercial IgG were tested for antibodies to GBS types Ia, Ib, II and III. The levels of antibodies in the different batches varied widely (fig. 1): type Ia between 1,260 and 3,800; Ib: 1,680–4040; II: 1,210–4,060; and III: 640–4,000 CPM. A correlation was found between the antibody levels against type Ia and Ib in the preparations (correlation coefficient $r = 0.22$; $p < 0.05$). The correlation coefficients with regard to other combinations of type antigens were not significant.

Seven batches contained antibody levels above 3,000 against two different types: two batches against the combination Ia/Ib and two against Ib/III; and the other three batches against the combinations Ia/III, Ib/II and II/III, respectively.

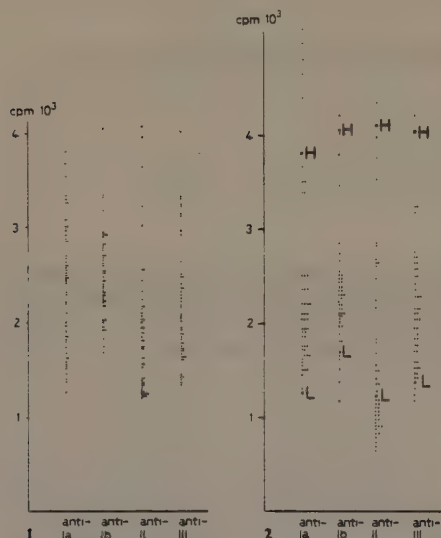


Fig. 1. Antibody levels against surface antigens of GBS types Ia, Ib, II and III in 53 different commercial IgG preparations. Ordinate: antibody levels in cpm.

Fig. 2. The content of anti-GBS antibodies in 50 blood donor sera, compared to the commercial IgG preparations containing the highest (H) and lowest (L) antibody level, respectively, against the four different types. The test tubes contained 0.85 mg commercial IgG and in mean 0.33 mg donor IgG under the conditions used. Ordinate: antibody levels in cpm.

The range of GBS antibody levels found in the commercial IgG was compared to the levels found in 50 different blood donor sera (fig. 2). The blood donor sera contained between 6.5 and 15 g/l of IgG (mean 10.7 g/l). The content of antibodies in 0.33 ml donor sera (in mean corresponding to 0.32 mg IgG) were compared to the results obtained with 0.85 mg of each of the commercial IgG preparations. Although the commercial IgG test preparations contained 2.6 times more IgG than the donor sera under these conditions, most donor sera fell within the range of the commercial IgG preparations.

Discussion

The antibody levels against GBS types Ia, Ib, II and III were found to vary greatly in the different

batches of commercial IgG tested (fig. 1). High levels against all types of GBS were not found in any of the batches, but seven batches contained levels above 3,000 against at least two of the four types. The correlation found between the antibody levels against types Ia and Ib was expected since these types share cross-reacting antigens [10].

Comparison of the GBS antibody levels in the commercial IgG preparations and in human blood donor sera showed that the commercial IgG contained approximately 2.6 times less type-specific antibody IgG than expected. This finding cannot be explained by differences in GBS spreading among the US donors from which the commercial IgG was taken and the Swedish blood donors, since the GBS carrier frequencies are similar in the two countries [2, 7].

The question arises if the relative lack of antibodies against GBS-type antigens in the commercial preparations might be due to the manufacturing procedures. *Oxelius* [12] has shown that Cohn fraction II contains smaller amounts of IgG1, IgG2 and IgG4 than those found in normal serum pools. Since antibodies to carbohydrate antigens belong mainly to the IgG2 subclass [13, 14, 16], it is strongly indicated that the Cohn-fractionation procedure causes a relative loss of antibodies against bacterial carbohydrate antigens. This can be compensated by administration of higher doses of gammaglobulin to, e.g., agammaglobulinemic patients. In the case of mass prophylaxis against GBS neonatal disease, however, the possibility of making better use of the GBS antibodies in the raw material for manufacturing of IgG preparations should be considered. We conclude that the commercial IgG now available is not optimal for passive immunization against GBS neonatal disease.

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OBSTETRICAL CARE IN FUTURE PREGNANCIES AFTER FETAL LOSS IN GROUP B STREPTOCOCCAL SEPTICEMIA. A PREVENTION PROGRAM BASED ON BACTERIOLOGICAL AND IMMUNOLOGICAL FOLLOW-UP

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CHRISTENSEN, K.K., DAHLANDER, K., LINDÉN, V., SVENNINGSSEN, N. and CHRISTENSEN, P. (1981): Obstetrical care in future pregnancies after fetal loss in Group B streptococcal septicemia. A prevention program based on bacteriological and immunological follow-up. *Europ. J. Obstet. Gynec. reprod. Biol.*, 12/3, 143–150.

Among 22 mothers of infants infected with group B streptococci (GBS), 19 showed markedly low levels of antibodies against the infecting type. Three of the patients with low antibody levels went through a new pregnancy within 1 yr after they had lost an infant (2 patients) or experienced fetal death due to GBS (1 patient). They were still urogenital carriers of the type of GBS causing the previous infection, and their serum levels of type-specific antibodies remained low. All three went through a successful pregnancy following a prevention program comprising antibiotic treatment from the 28th wk of pregnancy.

fetal death; urogenital carriers of GBS; penicillin

INTRODUCTION

Early onset neonatal infection with group B streptococci (GBS) is one of the most serious and most frequently occurring infections in newborns (Eickhoff et al., 1964; Franciosi et al., 1973; Baker, 1977). The mortality rate is high, 50–75% (Eickhoff et al., 1964; Franciosi et al., 1973; Baker, 1977). The disease can affect infants of any gestational age, from the most immature to apparently healthy infants born at term. This is in contrast to other neonatal diseases with high mortality rates, e.g., idiopathic respiratory distress syndrome, or intracerebral hemorrhage, which occur almost exclu-

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sively among prematures. Furthermore, GBS can lead to intrauterine fetal death (Bergqvist et al., 1978) and may give rise to fetal distress during labor (Christensen et al., 1980b).

The early onset form of GBS infection may develop precipitately, and antibiotic therapy is often begun too late. Therefore, prophylactic administration of antibiotics to pregnant carriers of GBS or their infants has been tried (Siegel et al., 1980). Routine administration of parenteral penicillin at birth reduces the frequency of neonatal GBS infection, whereas diseases caused by other pathogens increase in frequency (Siegel et al., 1980). These findings do not speak in favor of generalized prophylactic measures in GBS carriers.

It has been shown that mothers of GBS-infected infants lack type-specific serum antibodies against the type of GBS infecting their infants (Baker and Kasper, 1976; Hemming et al., 1976; Christensen et al., 1980a; Vogel et al., 1980). However, not all infants of GBS carriers who lack type-specific antibodies become infected (Baker and Kasper, 1976; Hemming et al., 1976; Christensen et al., 1980a; Vogel et al., 1980), indicating that other factors may predispose to the disease. Despite our deficient knowledge about these factors it is possible to distinguish a group of women at high risk among the GBS carriers with low-type-specific antibody levels. This group consists of pregnant women who (1) have previously given birth to a baby with serious GBS infection or experienced intrauterine fetal death due to GBS; (2) are still carriers of the type of GBS causing the previous infection; and (3) have unaltered low levels of antibody against this type of GBS.

The present paper describes the successful obstetrical care of 3 women belonging to the risk group. Briefly, these patients were given antibiotics from the 28th wk of pregnancy and the treatment was continued in the baby after birth.

MATERIALS AND METHODS

Patients giving birth to infants with serious GBS infections

Specimens from 22 infants with serious infections and from their respective mothers were obtained from various parts of Sweden during the period of January, 1978 to December, 1980. Twenty of the infants fell ill within 48 h after birth, with GBS septicemia and/or meningitis (early onset type) while 2 fetuses were infected in utero, leading to stillbirth in pregnancy during weeks 23 and 35, respectively. Six (30%) of the 20 infants with early onset disease died from the infection.

GBS strains were isolated from blood and/or cerebrospinal fluid specimens in the 20 infants who contracted early onset GBS neonatal infection. The GBS strains from the fetuses which died in utero were isolated as the sole microorganism from all inner organs during sterile autopsy, performed as described by Christensen et al. (Unpubl. obs.). None of these fetuses showed malformations. GBS strains were also isolated from the urethra and/or cervix of each mother, and a serum specimen was obtained for quantitation of GBS antibodies.

Prospective study of pregnancy and delivery in 3 mothers who had previously given birth to infants with serious GBS infections or experienced intra-uterine fetal death due to GBS

Obstetrical and neonatal care. The antenatal care of the 3 mothers comprised clinical examinations, blood pressure measurements, laboratory tests (hemoglobin, Wasserman, rubella-antibody titer and tests for albuminuria and glycosuria) including bacterial culture of the urine, fetal heart rate (FHR) and fundal height measurements. Ultrasound scanning was performed at least three times during the pregnancy, in weeks 8, 18 and 32. FHR recordings were obtained weekly from the 28th wk. During the first trimester, urethral and cervical specimens for culture of GBS using a selective broth (Baker et al., 1973) and serum specimens for antibody quantitation against GBS were obtained on one occasion. The GBS cultures were repeated from the 28th wk of pregnancy. Specimens were also taken weekly (using sterile cotton swabs) from the urethra and the cervix for culture of other potentially pathogenic bacteria (β -streptococci of other groups, *S. aureus*, *Enterobacteriaceae*, *Pseudomonas*, *Listeria monocytogenes*, *H. influenza*, etc.).

Treatment with V-penicillin (0.8 g twice daily) was started in the 28th wk and continued until delivery. If spontaneous labor did not occur at term, delivery was induced by amniotomy and oxytocin stimulation. During labor ampicillin was given intravenously (2 g every 6 h). The fetus and mother were supervised with electronic fetal monitoring.

Immediately after birth the infant was examined by a neonatologist, and specimens for bacteriological examinations were taken from the external ear, the throat and the umbilicus. The infant was transferred to the neonatal intensive care unit for observation during the first days of life. Treatment with cefalexin was started and continued for 4 wk, after which the infant was examined again.

Four weeks after birth, specimens for cultivation of GBS were again taken from the mother's urethra and cervix.

Microbiological examinations

The cultures and identification tests for potentially pathogenic bacteria were performed as described by Christensen et al. (Unpubl. obs.). Identification, grouping and typing of GBS were performed as described elsewhere (Christensen et al., 1980a). Antibodies to GBS types Ia, Ib, II and III were measured with radiolabelled protein A (Christensen et al., 1980a). In brief, the serum to be tested was absorbed with type II GBS before determination of antibodies against types Ia, Ib and III, and with type III GBS, when antibodies to type II were being measured. The absorbed serum was mixed with a standard suspension of the type of GBS to which antibodies were to be measured. The quantity of antibodies bound to the surface of the bacteria after washings was determined with protein A which reacts with the Fc part of human IgG subclasses 1, 2 and 4 (Kronvall and Williams, 1969). The antibody level was expressed as the radioactive protein A in counts per minute (cpm) bound to the bacteria after coating with serum.

The antibody levels measured were compared with those in sera from mothers found to be harboring types Ia, Ib, II and III in the urogenital tract and who gave birth to infants without signs of infection. The antibody levels among the controls were as follows. Against type Ia: 8100–17 000 cpm (mean 11 100 cpm); against type Ib: 17 000–28 000 cpm (mean 20 760 cpm); against type II: 12 500–23 000 cpm (mean 18 750 cpm); and against type III: 9000–31 000 cpm (mean 17 300 cpm). These results were based on tests of sera from 10 individuals for each type of GBS.

RESULTS

Serum levels of antibodies in 22 mothers giving birth to infants with early onset GBS diseases, or who had fetuses who died in utero due to GBS infection

GBS type Ia was isolated from 4 of the 20 infants with early onset septicaemia and/or meningitis, type Ib from 4, type II from 2 and type III from 10 infants. Types II and III were isolated from each of the intrauterine-death fetuses.

All mothers harbored the same type of GBS in the urogenital tract as iso-

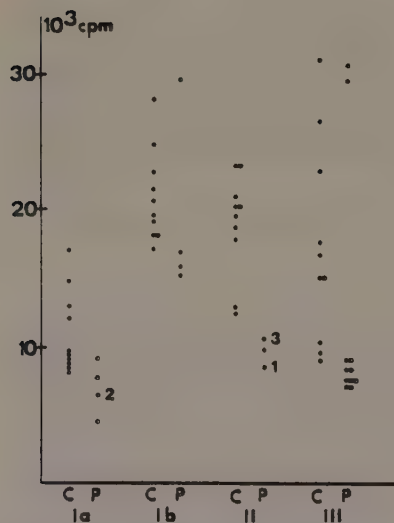


Fig. 1. Test of sera from mothers of infants with GBS sepsis and/or meningitis (P) and sera from mothers of infants who were healthy in the neonatal period (C). Each maternal serum was tested against the type of GBS infecting the corresponding infant, or that found in the urogenital tract of each mother giving birth to a noninfected infant. Ordinate: uptake of radiolabelled protein A on the bacteria after addition of serum. Ia, Ib, II and III indicate the bacteria against which antibodies were measured. 1, 2 and 3 indicate the serum levels of antibody in patients no. 1, 2 and 3, respectively.

lated from their respective infant or fetus; one mother carried an additional type.

The serum levels of antibodies against the type of GBS isolated from each individual patient are given in Fig. 1. All mothers of infants (or fetuses) infected with type Ia or type II had very low levels of type-specific antibodies against the respective type, when compared with the controls. Four of the 5 mothers of Ib-infected infants had low levels of type-specific antibodies against this type. The mother with a high level of antibodies to type Ib suffered from juvenile diabetes. Nine of 11 mothers of infants acquiring type III infections had low levels of antibodies against type III, while 2 mothers had relatively high levels. However, both infants had umbilical catheters inserted immediately after birth; of these, one infant was born in week 29 and the mother of the other infant was heavily ABO-immunized resulting in hemolytic disease in the infant.

Outcome of pregnancies following fetal wastage in GBS infection

Three of the patients listed above went through a new pregnancy after loss of an infant (2 patients) or a fetus (1 patient) due to GBS infection, and were checked and treated according to the prevention program.

CASE REPORTS

Case no. 1

This patient was a 28-yr-old gravida 4. She had experienced one spontaneous abortion in week 9 of pregnancy and gave birth to a healthy infant 3 years ago. One year before the present pregnancy she gave birth to an infant who died after 36 h of a type II GBS infection. Her serum antibody level against type II was very low immediately after the delivery (8100 cpm).

During the present pregnancy she was still a carrier of GBS type II in the urogenital tract and her antibody level was unchanged. Specimens taken from the urogenital tract during penicillin treatment showed a growth of GBS type II on one occasion after 3 wk of treatment; no other potentially pathogenic bacteria were isolated. Labor was induced in the 38th wk, and she gave birth to a boy of 2800 g, who was healthy and developed normally. Upon examination 4 wk after the delivery, GBS type II was again found in the urethral and cervical specimens.

Case no. 2

This patient was a 29-yr-old gravida 2. After normal development of the first pregnancy, she delivered a baby who died after 6 h of a type Ia GBS sepsis. GBS type Ia was also isolated from the urogenital tract of the mother, and she had low levels of antibodies against type Ia (6300 cpm) which remained low during the following pregnancy. During this pregnancy GBS type Ia could be isolated from the urogenital tract before treatment with penicillin was started, whereas the specimens obtained during the treatment were all negative. In the 39th week of pregnancy the patient gave birth to a

girl of 4200 g. The baby remained healthy throughout the neonatal period. Four weeks after the delivery GBS type Ia was again found in the urogenital tract of the mother.

Case no. 3

This patient was a 37-yr-old gravida 3 (one healthy infant). One year before the present pregnancy, she had experienced a late spontaneous abortion (week 23). Specimens from all inner organs of the fetus obtained at sterile autopsy revealed growth of GBS type II. No other microorganisms were isolated (including search for viruses, chlamydia and mycoplasmas). Her antibody level against type II GBS was low (1200 cpm) and remained so during the following pregnancy. GBS type II was also isolated from the patient's urogenital tract. Early in the present pregnancy she still carried GBS type II. GBS type II was isolated once during treatment and again after delivery. In the 34th wk, *E. coli* could be isolated from the cervical specimen, but apart from that, all specimens were negative.

DISCUSSION

In this investigation, a risk group of pregnant GBS-carriers was defined, and a program for prevention of serious fetal and neonatal GBS infection was instituted. The risk group was characterized by the following conditions. (1) The patient had previously given birth to an infant who had contracted GBS sepsis and/or meningitis, or she had experienced intrauterine fetal death due to GBS; (2) she was still a urogenital carrier of the type of GBS causing the previous infection; and (3) her serum antibody level against this type remained low. It follows that correct diagnosis of neonatal or fetal death is mandatory in order to detect members of this group, and that typing of isolated GBS is needed to monitor colonization and development of antibodies during future pregnancies.

In order to determine risk levels of GBS antibodies, we tested the sera from 22 mothers who had given birth to an infant with early onset GBS infection or who had experienced intrauterine fetal death due to GBS. The mortality rate in early onset disease among the infants was 30% (6 out of 20). In addition, 2 patients had a miscarriage due to antenatal infection. Nineteen of the 22 patients had low levels of antibodies against the infecting type of GBS, when compared with urogenital carriers of GBS having given birth to noninfected infants (Fig. 1). This observation, confirming our previous findings in 7 patients (Christensen et al., 1980a), is in agreement with several other investigations, in which a variety of antibody-quantitation techniques were used (Baker and Kasper, 1976; Hemming et al., 1976; Vogel et al., 1980). The 3 patients with high GBS antibody levels (Fig. 1) illustrate that other obstetrical and neonatal complications may overcome the immunity barrier.

Three of 8 patients who had lost their infant or fetus, were pregnant again within 1 yr. It was found that all 3 were still carriers of the type of GBS

causing the fatal infection. Furthermore, their serum levels of type-specific antibodies remained low. Thus all 3 fell within the risk group definition. Considering the unanimous reports on the importance of type-specific antibodies, we found it ethically impossible to design any form of placebo-controlled study on this patient category.

The purpose of the prevention program was to suppress GBS in the maternal urogenital tract and thereby minimize the risk of contamination of the newborn. Furthermore, we wanted to achieve a sufficiently high concentration of antibiotic in the fetus, in order to inhibit infection in utero; also, antenatal FHR recordings were taken weekly to discover intrauterine distress. One well-known risk of protracted administration of antibiotics is the possibility of selecting resistant strains. Penicillin was chosen for administration during pregnancy because of its low toxicity and the scant likelihood of selecting resistant strains, such as *Klebsiella* or *Pseudomonas*. Urethral and cervical specimens were taken weekly during pregnancy to ensure that no super-infection developed. With the exception of GBS, *E. coli* was the only potentially pathogenic bacterium isolated during treatment (found on one occasion in week 34 from the cervix of patient no. 3). Our observations demonstrate that it is not possible to eradicate GBS from the urogenital tract by administering penicillin. GBS was isolated on one occasion from each 2 patients during treatment. Furthermore, 4 wk after delivery, all 3 patients were carriers of the type of GBS isolated at the beginning of the pregnancy, although they denied that sexual intercourse had occurred since the delivery.

Ampicillin was given intravenously during the delivery in order to achieve a high concentration of antibiotic in the fetus (penicillin given intravenously would probably be equally protective). The administration of antibiotic to the newborn was continued, using cefalexin, which was chosen because of occasional outbreaks of *Klebsiella* on the neonatal ward. The infants were all culture-negative at birth and at 4 wk of age. No side-effects of the treatment of the mothers or infants were noted. Thus, the prevention program seemed to have been a success in the 3 patients.

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